



RESEARCH HIGHLIGHT

m⁶A reader suppression bolsters HSC expansionXinxin Huang¹ and Hal E. Broxmeyer¹Cell Research (2018) 28:875–876; <https://doi.org/10.1038/s41422-018-0081-z>

The functional importance of N⁶-methyladenosine (m⁶A) modification in human health and disease has recently entered the research spotlight. In a recent paper published in *Cell Research*, Li et al. report a remarkable finding that deficiency in m⁶A reader Ythdf2 results in a striking expansion of mouse and human hematopoietic stem cells.

RNA is made up of four nucleotides: A, C, G and U. Information on RNA molecules relies on their core sequence, and also their chemical modifications. RNA chemical modifications play important roles in various steps of RNA processing, degradation, translation, and thus in the regulation of gene expression. Amongst the more than 100 different chemical modifications on RNA identified, N⁶-methyladenosine (m⁶A) is the most abundant epigenetic mark on eukaryotic messenger RNAs (mRNAs). The m⁶A modification has been implicated in regulating a range of biological processes, and dysregulation of m⁶A modification has been associated with many human diseases including cancer and obesity.¹

Recent research has identified enzymes specifically involved in adjusting m⁶A methylation levels and their functional outcomes; this has been considered another layer of epigenetic control similar to DNA and histone modifications, and has increased our knowledge of epitranscriptomics.² The m⁶A methyltransferase complex, or the “writer”, consists of Methyl transferase-like 3 and 14 (METTL3 and METTL14) with the cofactor Wilms tumor 1 associated protein (WTAP). METTL3 and METTL14 play critical roles in both normal hematopoiesis and leukemogenesis.³ On the other hand, fat mass and obesity associated protein (FTO) and ALKBH5 act as demethylases to erase m⁶A modifications, and thus maintain a dynamic balance of m⁶A levels. The function of FTO has been associated with body fat homeostasis, and recently with oncogenesis of acute myeloid leukemia.³

The m⁶A modification is recognized by its readers, YTH domain family proteins, amongst which YTHDF2 destabilizes m⁶A-modified mRNAs after binding within the consensus sequences.² A recent study reports that YTHDF2 deficiency results in female infertility in mice, and YTHDF2 is maternally required for oocyte maturation.⁴ However, the physiological role of YTHDF2 in hematopoietic stem cell (HSC) biology was still unknown. In the present study, Li et al. observe a significant increase in numbers of HSCs in *Ythdf2* conditional knockout mice compared to wild-type littermates.⁵ The *Ythdf2*-knockout mice showed no difference in numbers of hematopoietic progenitors and mature lineage cells, suggesting that *Ythdf2* deficiency in hematopoietic lineages specifically increases HSC numbers. Limiting dilution in vivo engraftment assay revealed a 2.2-fold increase in competitive repopulating units (a measure of the numbers of functionally active HSCs) in *Ythdf2*-knockout, compared with control, bone marrow cells. Importantly, *Ythdf2*-knockout HSCs manifested

normal lineage differentiation, with no apparent signs of hematological malignancies.

Another interesting observation is that the m⁶A modifications are enriched in mRNAs of several transcriptional factors that are crucial for self-renewal of HSCs, such as *Gata2*, *Tal1*, *Etv6* and *Stat5*.⁵ *Ythdf2* deficiency resulted in extended stability of these m⁶A-marked mRNAs and elevated expression of these transcriptional factors (Fig. 1). Decapping of mRNA is an important step in mRNA decay, and *Dcp1a* encodes an mRNA decapping enzyme. Using fluorescence in situ hybridization, Li et al. were able to demonstrate co-localization of *Tal1* mRNA, *Dcp1a* and *Ythdf2* in wild-type cells but not in *Ythdf2*-knockout cells. They also showed that knockdown of *Tal1* suppressed the increased engraftment of *Ythdf2*-knockout HSCs, supporting their conclusion that upregulation of HSC self-renewal factors accounted for the HSC expansion phenotype in *Ythdf2*-knockout mice.⁵

Human cord blood (CB) is an alternative source of HSCs for transplantation in clinical applications.^{6,7} However, limited numbers of HSCs in single CB collections have hampered wider use of CB in patients with malignant and nonmalignant disorders. While progress has been made to expand CB HSC numbers ex vivo,⁷ additional means are still needed to meet clinical demands. In an attempt to reveal whether the function of m⁶A and *Ythdf2* in HSCs would be conserved in human cells, Li et al. first performed m⁶A RNA-seq in human CB CD34⁺ cells and found that many transcriptional factors involved in HSC self-renewal were marked by m⁶A modifications. They were then able to show that knockdown of *YTHDF2* resulted in increased HSC numbers with long-term engraftment capability in vivo in immune-deficient mice. Thus, the exciting work by Li et al. adds to our understanding of m⁶A and *YTHDF2* function in HSC expansion in both mouse and human (Fig. 1).

Recent progress has shed new light on enhancing CB transplantation efficacy by several approaches: collecting and processing HSCs at physiological low oxygen levels to prevent their differentiation and increase numbers;⁸ modifying membrane expression of a homing receptor, CXCR4;⁹ modulating epigenetic regulations to promote the HSC homing process;^{10,11} and by manipulating the cellular metabolism of HSCs to ex vivo expand them.¹² Thus, it would be interesting to see whether the effects will be even greater by combining several different approaches for both enhanced HSC homing and ex vivo expansion with *YTHDF2* inhibition.

To conclude, Li et al. present important information towards further understanding the physiological impact of m⁶A modification and HSC biology. Looking to the future, the new findings that HSC expansion can be achieved by *Ythdf2* suppression open up a new and potent direction to simultaneously target multiple key transcriptional factors for HSC self-renewal, and to engineer HSCs

¹Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, USA
Correspondence: Hal E. Broxmeyer (hbroxmey@iupui.edu)

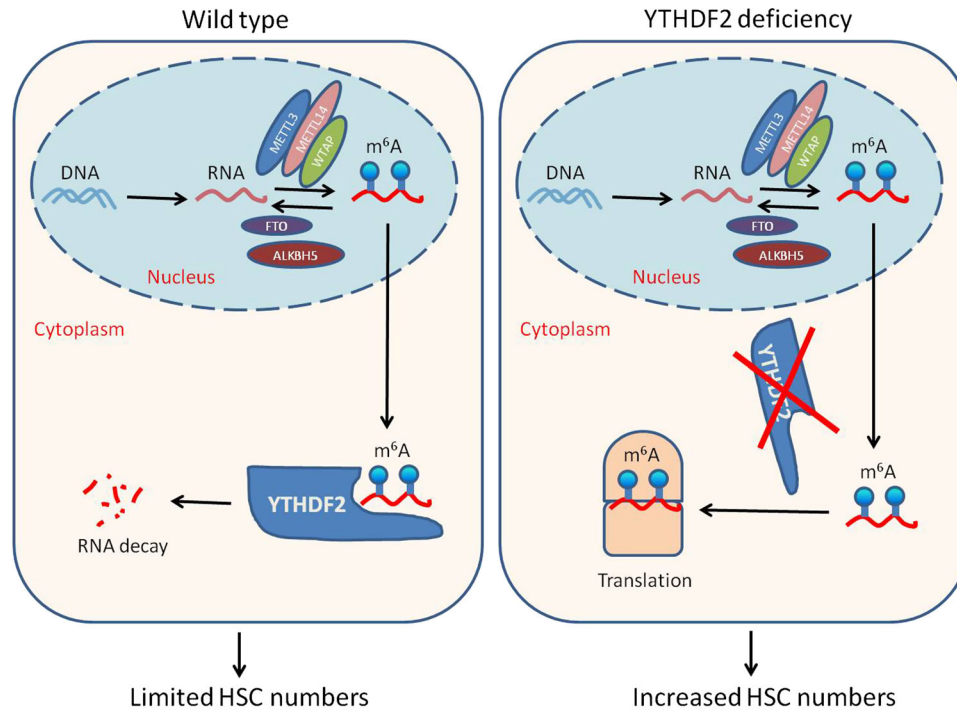


Fig. 1 In wild-type cells, m⁶A reader Ythdf2 recognizes m⁶A-modified RNAs and thus targets these RNAs for degradation (left). In the present study, Li et al. demonstrate that Ythdf2 deficiency results in increased stability of m⁶A-modified RNAs, leading to elevated expression of HSC self-renewal factors and increased HSC numbers (right)

for therapeutic advantage, effects that may be relevant beyond the enhancement of hematopoiesis. Identifying specific small-molecule inhibitors for Ythdf2 may provide a promising way to enhance HSC transplantation, especially with CB cells, and to also benefit HSC-based gene therapy.

REFERENCES

1. Fu, Y. et al. *Nat. Rev. Genet.* **15**, 293–306 (2014).
2. Roundtree, I. A. et al. *Cell* **169**, 1187–1200 (2017).
3. Deng, X. et al. *Cell Res.* **28**, 507–517 (2018).
4. Ivanova, I. et al. *Mol. Cell* **67**, 1059–1067 (2017).
5. Li, Z., et al. *Cell Res.* <https://doi.org/10.1038/s41422-018-0072-0> (2018).
6. Ballen, K. K., Gluckman, E. & Broxmeyer, H. E. *Blood* **122**, 491–498 (2013).
7. Broxmeyer, H. E. *Transfus. Apher. Sci.* **54**, 364–372 (2016).
8. Mantel, C. R. et al. *Cell* **161**, 1553–1565 (2015).
9. Capitano, M. L. et al. *Stem Cells* **33**, 1975–1984 (2015).
10. Huang, X. et al. *Nat. Commun.* **9**, 2741 (2018).
11. Guo, B. et al. *Nat. Med.* **23**, 424–428 (2017).
12. Guo, B. et al. *Nat. Med.* **24**, 360–367 (2018).