YTHDF3 as a new player in hematopoietic stem cell regulation

Xinjian Mao[1] and Linheng Li[1,2]
[1]Stowers Institute for Medical Research and [2]Department of Pathology and Laboratory Medicine, University of Kansas, Kansas City, MO, USA
E-mail: LIL@stowers.org
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Hematopoietic stem cells (HSC) are characterized by self-renewal and multipotent differentiation potential, and are required to maintain the hematopoietic system throughout life. Since HSC transplantation is a life-saving treatment for various hematopoietic disorders, dissecting the mechanisms underlying how intrinsic programs and extrinsic niche signals are orchestrated to regulate HSC *in vivo* would enable development of new methods for *ex vivo* HSC expansion, and would potentially benefit clinical treatment for blood disorders.

N6-methyladenosine (m^6A) is the most prevalent messenger RNA (mRNA) modification in mammals.[1] The proteins involved in the deposition, removal, and execution of the m^6A modification are referred to as m^6A 'writers', 'erasers' and 'readers', respectively.[2] Over the last decade, the m^6A modification has been demonstrated to play essential and broad roles in the regulation of RNA stability and translation in various types of cells, although the detailed mechanisms require further exploration and seem to be cell- and context-dependent.[2,3] In recent years, several studies have revealed that deficiency of the m^6A writer *Mettl3* or *Mettl14* impairs self-renewal and differentiation capacity of HSC.[4-6] In contrast, two groups independently discovered that loss of *Ythdf2* promotes HSC expansion and regeneration.[7,8] Given that the m^6A readers are the primary component responsible for exerting the function of m^6A-containing RNA deposited by METTL3, an important question is: which readers are responsible for the characteristics of *Mettl3*$^{-/-}$ HSC?

As described in their paper in this issue of *Haematologica*, to test the hypothesis that other readers in the cytoplasm may be responsible for the hematopoietic phenotype in *Mettl3*$^{-/-}$ mice, Zhang *et al.* first investigated the hematopoietic system in *Ythdf1*$^{-/-}$ and *Ythdf3*$^{-/-}$ mice.[9] Both the frequency and the absolute number of HSC in *Ythdf3*$^{-/-}$ mice, unlike in *Ythdf1*$^{-/-}$ mice, were significantly increased when compared with those in wild-type mice. Of note, the magnitude of the increase in HSC in *Ythdf3*$^{-/-}$ mice was much smaller than that in *Mettl3*$^{-/-}$ mice. No other significant differences in hematopoietic cells were found between *Ythdf3*$^{-/-}$ and the littermate mice. In contrast, bone marrow cellularity and hematopoietic cells declined markedly in *Mettl3*$^{-/-}$ mice. Consistent with the severely impaired reconstitution ability of *Mettl3*$^{-/-}$ HSC, the *Ythdf3*$^{-/-}$ HSC exhibited poor reconstitution capacity with similar multilineage differentiation, comparable homing capacity, higher sensitivity to replication stress, and diminished protein synthesis.

Given that *Ythdf3*$^{-/-}$ HSC resemble *Mettl3*$^{-/-}$ HSC to some extent, and that targets of *Ythdf3* and *Ythdf2* overlap partially, Zhang *et al.* reasoned that *Ythdf3* may mediate HSC via reversely regulating the targets of *Ythdf2*. Among six mRNA previously identified to be increased in *Ythdf2*$^{-/-}$ HSC,[8] only *Ccnd1* was decreased in *Ythdf3*$^{-/-}$ HSC. Based on these findings, the next question to be explored in the future is: how do YTHDF2 and YHTDF3 orchestrate the regulation of *Ccnd1* expression and in what context do YTHDF2 and YTHDF3 each dominate such regulation? Knockdown assays confirmed that the protein level of *Ccnd1*, rather than the mRNA level, decreased significantly in both *Ythdf3* shRNA-carrying and *Mettl3* shRNA-carrying HSC. This result is congruent with the role of the m^6A modification in regulating gene expression at a post-transcriptional level. The 5' untranslated region of *Ccnd1* in the -180 to -184 region was identified as the major m^6A motif recognized by YTHDF3 and METTL3. YTHDF3 cooperated with PABPC1 and eIF4G2 to promote the translation of *Ccnd1*. Furthermore, knockdown of *Ccnd1* recapitulated the compromised reconstitution capacity of *Ythdf3*$^{-/-}$ HSC. On the other hand, forced expression of *Ccnd1* completely rescued the reconstitution potential of *Ythdf3* shRNA-carrying HSC, but not of *Mettl3* shRNA-carrying HSC. *Myc* has previously been reported to be an important target gene of *Mettl3*, and enforced expression of *Myc* rescued the lineage differentiation bias of *Mettl3*$^{-/-}$ HSC. To investigate the role of *Myc* in reconstitution potential, Zhang *et al.* overexpressed *Myc* in *Mettl3*$^{-/-}$ HSC and evaluated the engraftment capacity of these cells. The result showed that forced expression of *Myc* failed to rescue the reconstitution ability of *Mettl3*$^{-/-}$ HSC, although it consistently rescued the lineage differentiation bias of *Mettl3*$^{-/-}$ HSC. These data indicate that, besides *Ythdf3* and *Myc*, other unknown players are responsible for the characteristics of *Mettl3*$^{-/-}$ HSC. Could the other m^6A readers (e.g., YTHDC1 and YTHDC2) be the missing link?

The m^6A modification has recently been discovered to play a critical role in the regulation of HSC, although the mechanisms are still not fully understood. Zhang *et al.* were able to establish that the *Mettl3-Ccnd1-Ythdf3* axis regulates the characteristics of HSC. Their study uncovers the missing piece of the puzzle by revealing that the m^6A reader YTHDF3 is partially responsible for *Mettl3* deficiency in HSC, broadening the knowledge of how RNA m^6A components el-

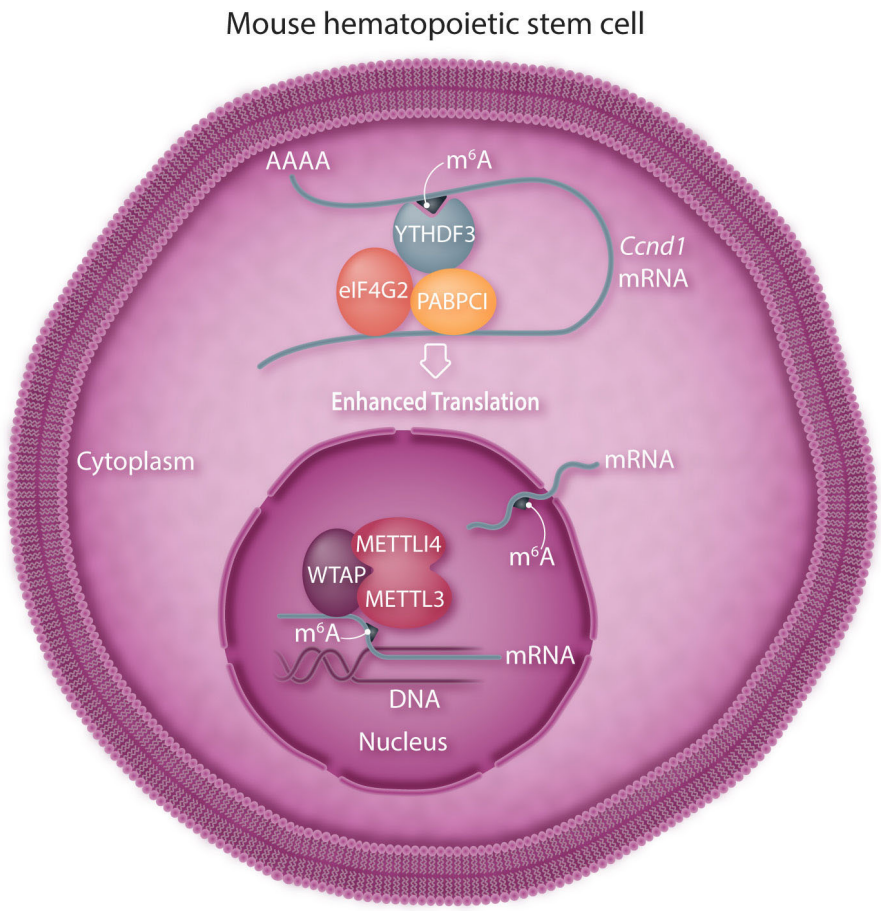

Figure 1. Proposed model of the *Mettl3* → RNA m^{6}A → *Ccnd1* → *Ythdf3* axis regulating the reconstitution capacity of mouse hematopoietic stem cells. METTL3, along with other components including METTL14 and WTAP, form a complex and deposit m^{6}A to *Ccnd1* mRNA in the nucleus. The m^{6}A-containing *Ccnd1* mRNA then travels from the nucleus to the cytoplasm for translation. YTHDF3 specifically recognizes the m^{6}A motif, mainly at the 5' UTR of *Ccnd1* mRNA in the region from -180 to -184, and increases the expression of *Ccnd1* by recruiting PABPC1 and eIF4G2 to enhance protein synthesis. The up- regulated expression of *Ccnd1* is responsible for YTHDF3-mediated reconstitution capacity of mouse hematopoietic stem cells.

egantly combine to regulate HSC (Figure 1). Based on this discovery, there are several questions to be explored further in the future: (i) Besides mRNA, METTL3 has recently been discovered to influence chromatin state and transcription of mouse embryonic stem cells via YTHDC1-mediated regulation of chromosome-associated regulatory RNA. Is this regulatory mechanism also applicable to adult stem cells, for example HSC? (ii) Do the regulatory mechanisms that govern mouse HSC properties, such as self-renewal and differentiation, also regulate similar properties in human HSC? Although a study previously demonstrated the opposite role of *Mettl3* in human hematopoietic stem and progenitor cells and in murine HSC, a series of experiments using purified human HSC, as opposed to human CD34$^+$ hematopoietic stem and progenitor cells, which only contain a very small population of HSC, is warranted to answer this question. (iii) Given that *Mettl3* deficiency leads to increased numbers and decreased differentiation of HSC, and that rapid differentiation of HSC in *in vitro* culture is a major challenge in the field, a pertinent question becomes: is it possible to inhibit METTL3 pharmacologically using small molecules to block differentiation of HSC in *ex vivo* cultures?

Disclosures

No conflicts of interest to disclose.

Contributions

XM wrote the editorial; LL supervised and edited it.

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