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by Xinjian Mao and Linheng Li

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YTHDF3 as a new player in hematopoietic stem cell regulation

Xinjian Mao¹, Linheng Li¹,²

Affiliations

¹Stowers Institute for Medical Research, Kansas City, MO, USA.

²Department of Pathology and Laboratory Medicine, University of Kansas, Kansas City, KS, USA.

Correspondence: Linheng Li, LIL@stowers.org

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Hematopoietic stem cells (HSCs) are characterized by self-renewal and multipotent differentiation potential, and thus, are required for maintaining the hematopoietic system throughout the lifespan. HSC transplantation is a life-saving treatment for various hematopoietic disorders. Therefore, dissecting the mechanisms underlying how intrinsic programs and extrinsic niche signals are orchestrated to regulate HSCs \textit{in vivo} would enable development of new methods for \textit{ex vivo} HSCs expansion, and would potentially benefit clinical treatment for blood disorders.

N6-methyladenosine (m6A) is the most prevalent mRNA modification in mammals.\textsuperscript{1} Regarding its role in the deposition, removal, and execution of m6A modification, the proteins correspondingly are referred to as m6A ‘writer’, ‘eraser’ and ‘reader’, respectively.\textsuperscript{2} Over the last decade, m6A 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.\textsuperscript{2,3} In recent years, several papers have revealed that the deficiency of m6A ‘writer’ \textit{Mettl3} or \textit{Mettl14} impaired self-renewal and differentiation capacity of HSCs.\textsuperscript{4-6} In contrast, two groups independently discovered that loss of \textit{Ythdf2} promotes HSC expansion and regeneration.\textsuperscript{7,8} Given that the m6A readers are the primary component responsible for exerting the function of m6A-containing RNA deposited by METTL3, an important question, then, is which readers are responsible for the characteristics of \textit{Mettl3}\textsuperscript{−/−} HSCs?

To test the hypothesis that other readers in the cytoplasm may be responsible for the hematopoietic phenotype in \textit{Mettl3}\textsuperscript{−/−} mice, Zhang et al. firstly investigated the hematopoietic system in \textit{Ythdf1}\textsuperscript{−/−} and \textit{Ythdf3}\textsuperscript{−/−} mice.\textsuperscript{9} Both the frequency and the absolute number of HSCs in \textit{Ythdf3}\textsuperscript{−/−} mice, in contrast with \textit{Ythdf1}\textsuperscript{−/−} mice, were significantly increased when compared with wild type mice (Fig. 1J). Of note, the magnitude of HSC increase in \textit{Ythdf3}\textsuperscript{−/−} mice was much lower than in \textit{Mettl3}\textsuperscript{−/−} mice (Fig. S6). No other significant difference in hematopoietic cells have been found between \textit{Ythdf3}\textsuperscript{−/−} and the littermate mice (Fig. 1 and S1). In contrast, bone marrow cellularity and hematopoietic cells declined prominently in \textit{Mettl3}\textsuperscript{−/−} mice (Fig. S6J). On the other hand, consistent with the severely impaired reconstitution ability of \textit{Mettl3}\textsuperscript{−/−} HSCs (Fig. 5H), \textit{Ythdf3}\textsuperscript{−/−} HSCs exhibited poor reconstitution capacity with similar multilineage differentiation, comparable homing capacity, higher sensitivity to replication stress, and declined protein synthesis (Fig. 2 and S2).

Given that \textit{Ythdf3}\textsuperscript{−/−} HSCs resemble \textit{Mettl3}\textsuperscript{−/−} HSCs to some extent, and that targets of \textit{Ythdf3} and \textit{Ythdf2} are partially overlapped, Zhang et al. reasoned that \textit{Ythdf3} may mediate HSCs via reversely regulating the targets of \textit{Ythdf2}. Among 6 mRNAs previously identified to be increased in \textit{Ythdf2}\textsuperscript{−/−} HSCs,\textsuperscript{8} only \textit{Ccnd1} was decreased in \textit{Ythdf3}\textsuperscript{−/−} HSCs (Fig. 3). Based on these findings, the next question to be explored in the future is how do YTHDF2 and YHTDF3 orchestrate to regulate \textit{Ccnd1} expression and in what context do
YTHDF2 and YTHDF3 each dominate such regulation? Knockdown assay further confirmed that protein level of Ccnd1, instead of mRNA level, significantly declined in both Ythdf3 shRNA-carrying and Mettl3 shRNA-carrying HSCs (Fig. 3). This result is congruent with the role of m6A modification on regulating gene expression at post-transcriptional level. In addition, the 5’ UTR of Ccnd1 in -180 ~ -184 region was identified as the major m6A motif recognized by YTHDF3 and METTL3 (Fig. 3 and S4). YTHDF3 cooperated with PABPC1 and eIF4G2 to promote the translation of Ccnd1 (Fig. 3 and S4). Furthermore, knockdown of Ccnd1 recapitulated the compromised reconstitution capacity of Ythdf3-/- HSCs. On the other hand, forced expression of Ccnd1 completely rescued the reconstitution potential of Ythdf3 shRNA-carrying HSCs (Fig. 4B and S5D), but not Mettl3 shRNA-carrying HSCs (Fig. 6). Myc has previously been reported as an important target gene of Mettl3, and enforced expression of Myc rescued the lineage differentiation bias of Mettl3-/- HSCs. To test the role of Myc in reconstitution potential, Zhang et al. overexpressed Myc in Mettl3-/- HSCs and evaluated their engraftment capacity. The result showed that forced expression of Myc failed to rescue the reconstitution ability of Mettl3-/- HSCs, although it consistently rescued the lineage differentiation bias of Mettl3-/- HSCs. These data indicate that, besides Ythdf3 and Myc, other unknown players are responsible for the characteristics of Mettl3-/- HSCs. Could the other m6A reader YTHDC1 and m6A erasers (e.g., FTO, ALKBH5) be the missing link? m⁶A modification has recently been discovered to play a critical role in regulation of HSCs, 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 HSCs. This study uncovers the missing piece of the puzzle by revealing that m6A reader YTHDF3 is partially responsible for Mettl3 deficiency in HSCs, broadening the knowledge of how RNA m6A components elegantly combine to regulate HSCs. Based on this discovery, there are several questions to be further explored in the future. 1) 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 RNAs. Is this regulatory mechanism also applicable to adult stem cells, for example HSCs? 2) Do the regulatory mechanisms that govern mouse HSC properties, such as self-renewal and differentiation, also regulate similar properties in human HSCs? Although a study has previously demonstrated the opposite role of Mettl3 in human HSPCs and in mouse HSCs, a series of experiments using purified human HSCs, instead of human CD34+ HSPCs that only contain a very small population of HSCs, is warranted to answer this question. 3) Given that Mettl3 deficiency leads to increased number and decreased differentiation of HSC, and that rapid differentiation of HSCs in in vitro culture is a major challenge in the field, a pertinent question becomes: is it possible to pharmacologically inhibit METTL3 using small molecules in order to block differentiation of HSCs in ex vivo cultures?
References


Figure 1: Proposed model of the Mettl3 → RNA m6A → Ccnd1 → Ythdf3 axis regulating the reconstitution capacity of mouse HSCs

METTL3, along with other components including METTL14 and WTAP, form a complex and deposit m6A to Ccnd1 mRNA in the nucleus. The m6A-containing Ccnd1 mRNA then travels from the nucleus to the cytoplasm for translation. YTHDF3 specifically recognizes the m6A motif, mainly at the 5' UTR of Ccnd1 mRNA in -180 ~ -184 region, 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 HSCs.