# Decoding m$^6$A, one reader at a time

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In this issue of *Haematologica*, Dang *et al.* demonstrated that the N$^6$-methyladenosine (m$^6$A) RNA methylation reader protein YTHDF3 protects hematopoietic stem cell (HSC) integrity under stress conditions.[1] The mRNA methylation pathway has emerged as one of the most important co-transcriptional regulatory processes that controls both normal and malignant hematopoiesis. A full accounting of the different m$^6$A mediators and their distinct roles remains to be established. The study by Dang *et al.* identifies a new reader that maintains HSC self-renewal under stress.

The m$^6$A mRNA modification is the most abundant co-transcriptional mark identified in eukaryotes.[2] A set of m$^6$A regulators, including "writers", "erasers" and "readers", have been identified to dynamically modify target mRNA and regulate their fate. The m$^6$A writer complex is composed of the core methyltransferase METTL3 which forms a heterodimer with METTL14 and adaptor proteins, including WTAP, RBM15 and ZC3H13. The erasers FTO and ALKBH5 are m6A demethylases that remove the m$^6$A mark, while the readers bind to m$^6$A-marked transcripts. The readers include forms of YTHDF (YTHDF1,2,3), YTHDC (YTHDC1,2), and IGF2BP (IGF2BP1,2,3).[2] These m$^6$A modulators have been implicated in mRNA stability, splicing, nuclear export, and translational efficiency.

Although RNA methylation has been implicated in a variety of tissues and diseases, there has been significant attention to both normal and malignant hematopoiesis. It was found that m6A writers METTL3 and METTL14 control myeloid differentiation and are essential for development of acute myeloid leukemia (AML).[2-4] Suggesting that the balance of m$^6$A methylation is important, the erasers FTO and ALKBH5 are both upregulated in several subtypes of AML and maintain leukemia stem cell activity.[2,5,6] Additionally, the reader YTHDF2 contributes to leukemia stem cell self-renewal and maintenance while the nuclear reader YTHDC1 forms condensates with m$^6$A marked transcripts and promotes AML cell survival.[7,8] Therefore, targeting the m$^6$A RNA methylation program has been proposed as a new therapeutic strategy against AML. Most excitingly, small molecules targeting m$^6$A regulators, including METTL3 and FTO, have now demonstrated therapeutic efficacy in AML models.[9,10]

While m$^6$A regulators seem to converge on an oncogenic role in AML, their roles in normal hematopoiesis are more complex. Loss of the m$^6$A writer METTL3 results in a symmetric commitment defect in HSC and a failure to differentiate, while deletion of the eraser ALKBH5 has minimal effects on normal hematopoiesis.[5,6,11] Suppression of the reader YTHDF2 promotes HSC self-renewal and results in HSC expansion at a young age but is detrimental to HSC function at an old age or under hematopoietic stress.[12] Inhibiting another reader, YTHDC1, results in defects in murine HSC self-renewal, but has minimal impact on CD34$^+$ human hematopoietic stem and progenitor cell (HSPC) survival.[8,13] Together, these studies imply that the m$^6$A program in normal blood cells and AML cells is cell-context dependent and requires further delineation.

In this issue of *Haematologica*, Dang *et al.* describe their investigation of role of the m$^6$A reader YTHDF3 in normal hematopoiesis.[1] YTHDF3 is the third member of the YTHDF family, sharing 65% protein sequence identity with both YTHDF1 and YTHDF2. These YTHDF paralogs share 90% homology in the YTH domain, which recognizes and binds to m$^6$A sites. Using *Ythdf3* knockout mice, Dang *et al.* first showed that YTHDF3 is dispensable for steady-state hematopoiesis. Germline *Ythdf3* knockout (KO) mice displayed comparable hematopoietic lineage compositions in the peripheral blood, bone marrow and spleen. Loss of YTHDF3 resulted in equivalent HSPC populations, and normal cell cycle and death percentages as compared to those in littermate controls.

To determine the role of YTHDF3 under stress conditions, the authors performed bone marrow transplantation assays. At transplantation, *Ythdf3* KO bone marrow cells engraft comparably to control cells. However, a significant reduction in engraftment in the HSPC compartment was observed. These data prompted the authors to further examine the impact of hematopoietic stress on *Ythdf3* KO bone marrow cells. Under a 5-fluorouracil challenge, which ablates mature myeloid cells and pushes dormant HSC into the cycle, mice that received *Ythdf3* KO bone marrow were more susceptible to death compared to the recipients of control bone marrow. Additionally, *Ythdf3* KO bone marrow cells exhibited a two-fold reduction in repopulating capacity in secondary transplantation experiments. This defect was also observed in HSC and without a lineage output bias in the *Ythdf3* KO cells. Together, these data suggest that YTHDF3 is required for HSC self-renewal and maintenance during stress hematopoiesis.

To address the molecular mechanism underlying the role of YTHDF3 in HSPC, the authors analyzed the publicly available YTHDF3 cross-linking immunoprecipitation (CLIP)-sequencing data in Hela cells. Given the established roles of *Foxm1*

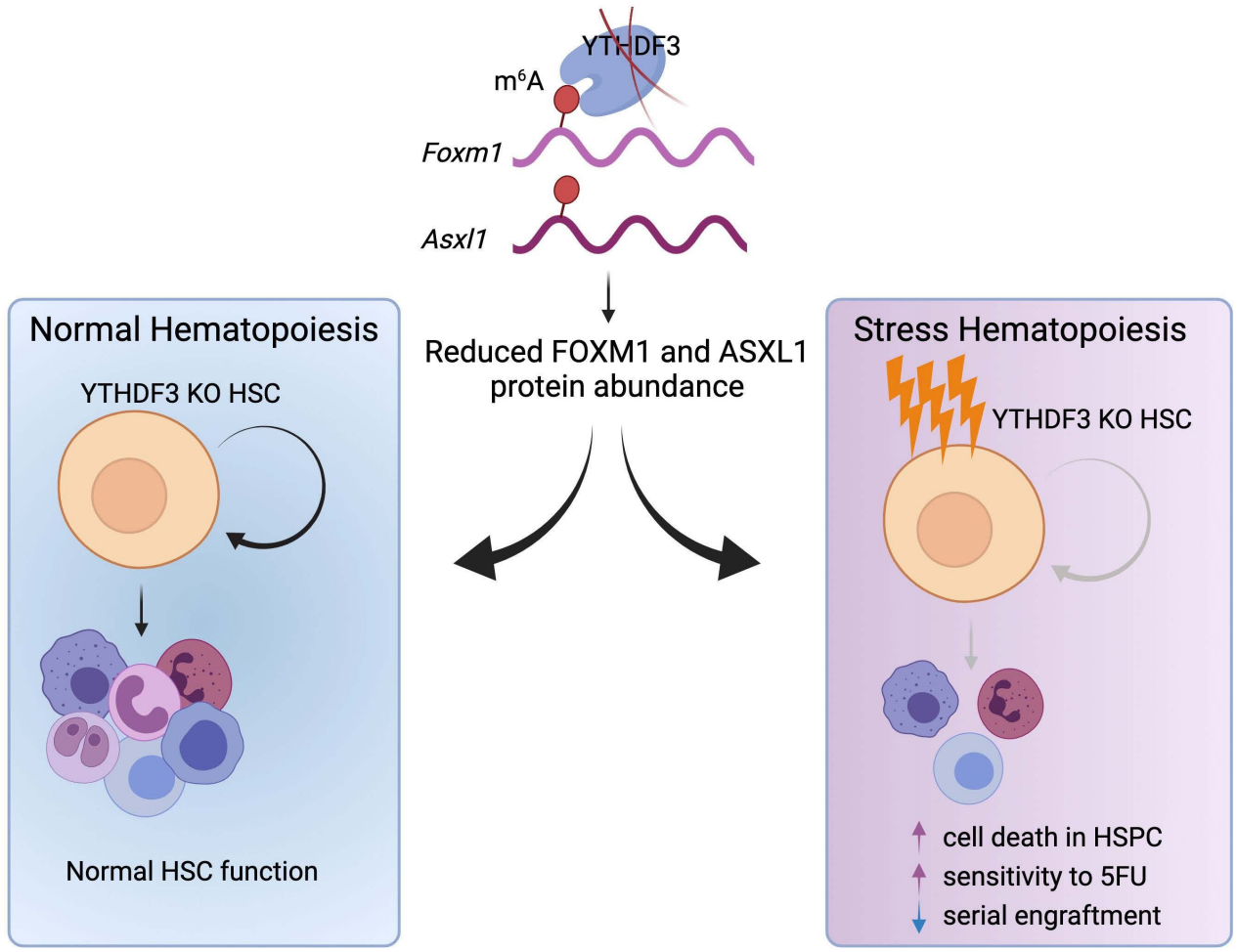


**Figure 1. YTHDF3 is required in hematopoietic stem cell maintenance under stress conditions.** KO: knockout; HSC: hematopoietic stem cells, HSPC: hematopoietic stem and progenitor stem cells., 5FU: 5-fluorouracil.

and *Asxl1* in HSC maintenance the authors focused on these transcripts. Both *Foxm1* and *Asxl1* transcripts were m$^6$A-modified and were direct binding targets of YTHDF3 in HPC-7 cells. Using either siRNA-mediated knockdown in HPC-7 cells or *Ythdf3* KO primary c-Kit$^+$ bone marrow progenitor cells, the authors found that YTHDF3 loss did not affect the mRNA levels but significantly reduced the protein abundance of FOXM1 and ASXL1. The authors then examined whether YTHDF3 regulates *Foxm1* and *Asxl1* translation and provided two pieces of evidence that support this hypothesis. First, they showed that the binding of the translation initiation factor EIF3A to the *Foxm1* and *Asxl1* transcripts was reduced upon YTHDF3 knockdown. Second, the half-life of FOXM1 and ASXL1 proteins remained unchanged upon YTHDF3 knockdown, suggesting the defect is during protein synthesis. Interestingly, this effect was not associated with YTHDF2 since YTHDF2 depletion had no effect on either RNA levels or protein abundance of these targets. These results suggest that YTHDF3, but not YTHDF2, promotes the translation of *Foxm1* and *Asxl1* in hematopoietic cells.

Both specific and shared functions for each YTHDF paralog have been proposed. Some studies suggested that YTHDF1 and YTHDF3 promote translation by recruiting translation initiation factors; while YTHDF2 enhances mRNA degradation, by recruiting the CCR4–NOT deadenylase complex.[2] Recently, a new model has been proposed, in which the YTHDF paralogs act redundantly on the same set of mRNA targets to regulate RNA stability.[14] This model is supported by YTHDF single knockouts and triple knockout studies in human Hela and mouse embryonic stem cells in which the YTHDF paralogs are all similarly expressed. However, whether the YTHDF paralogs also act redundantly in hematopoiesis remains to be tested. Thus, the paper by Dang *et al.* provides new insights into the role of another YTHDF paralog YTHDF3 in hematopoiesis. In contrast to YTHDF2, which can expand HSC numbers, YTHDF3 is only required during stress hematopoiesis. Moreover, the authors found that YTHDF3, but not YTHDF2, translationally regulates *Foxm1* and *Asxl1* transcripts. These results suggest that YTHDF3 and YTHDF2 may act on different sets of mRNA targets in HPC-7 cells and indicate that the YTHDF paralogs may act non-redundantly in hematopoiesis. Future studies are needed to globally compare the direct binding targets of the YTHDF paralogs in HSPC and their redundant and non-redundant functions.

Overall, the study by Dang *et al.* demonstrates a role for the m$^6$A reader YTHDF3 in HSC self-renewal and maintenance under stress conditions (Figure 1). It also implies cell-context dependency for individual YTHDF paralogs. Further studies on additional m$^6$A regulators and their downstream targets will uncover the complex m$^6$A network in normal and malignant hematopoiesis.

## Disclosures

*No conflicts of interest to disclose.*

## Contributions

*HL and MK both contributed to this editorial.*

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