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RNA methylation regulates hematopoietic stem/progenitor cell specification

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RNA methylation, conceptually similar to methylation of DNA and protein, has been identified in mRNAs and non-coding RNAs (ncRNAs) (Niu et al., 2013). In particular, N^6-methyl-adenosine (m^6A) is the most prevalent mRNA modification in eukaryotes (Jia et al., 2013). New insights into the biological functions of m^6A modification have been recently gained due to the rapid development of high-throughput sequencing technologies. mRNAs are methylated by the m^6A methyltransferase complex containing methyltransferase like 3 (METTL3), METTL14, and Wilms' tumor 1-associating protein (WTAP) (Liu et al., 2014; Ping et al., 2014; Schwartz et al., 2014; Wang et al., 2014a). This process can be reversed by the demethylases fat-mass and obesity associated protein (FTO) and alkylated DNA repair protein AlkB homolog 5 (ALKBH5) (Jia et al., 2011; Zheng et al., 2013). The methylated mRNAs are directly recognized by the YTH-domain containing family proteins (Wang et al., 2014a; Wang et al., 2015; Xiao et al., 2016), thus mediating a variety of RNA processing steps, such as mRNA splicing (Xiao et al., 2016), mRNA stability (Wang et al., 2014a), translation efficiency (Meyer et al., 2015; Wang et al., 2015; Zhou et al., 2015), microRNA processing (Alarcón et al., 2015a; Alarcón et al., 2015b), and transcriptional repression (Patil et al., 2016).

m^6A methylation/demethylation process has been identified to play crucial regulatory roles in a wide range of biological processes including circadian clock (Fustin et al., 2013), cellular resistance to UV damage (Xiang et al., 2017), obesity (Zhao et al., 2014), spermatogenesis (Zheng et al., 2013), embryonic stem cell (ESC) pluripotency (Batista et al., 2014; Wang et al., 2014b; Aguilo et al., 2015; Chen et al., 2015; Geula et al., 2015), maternal-to-zygotic transition (Zhao et al., 2017), sex determination (Haussmann et al., 2016; Lence et al., 2016), T cell homeostasis (Li et al., 2017), and plant shoot stem cell fate determination (Shen et al., 2016). However, due to the early lethality of knockout (KO) mice of m^6A methyltransferase catalytic subunit *Mettl3* (Geula et al., 2015), the exact function of m^6A modification in vertebrate embryogenesis *in vivo* remains elusive.

Hematopoietic stem/progenitor cells (HSPCs) are capable of self-renewal and differentiation into all blood cell lineages. Due to these unique properties, HSPCs attract keen interest in the field of regenerative medicine. However, the limited availability of donors to provide sufficient HSPCs for clinical applications makes it imperative to produce HSPCs *ex vivo* or *in vitro*. Unraveling the mechanisms underlying the HSPC generation *in vivo* is a prerequisite to achieve this goal. In vertebrate embryos, HSPCs are derived from hemogenic endothelium (HE), a subset of endothelial cells (ECs) in the ventral wall of dorsal aorta (DA), through a process named endothelial-to-hematopoietic transition (EHT) (Bertrand et al., 2010; Boisset et al., 2010; Kissa and Herbomel, 2010). Hemogenic endothelial cells must tune down the arterial program, including the expression of Sox17

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(Lizama et al., 2015), Notch1 (Richard et al., 2013; Zhang et al., 2015), and ERK1/2 (Zhang et al., 2014) after artery-vein specification, to acquire the hematopoietic fate during this transition. However, the detailed regulatory mechanism underlying HSPC specification remains unclear.

Recent studies have suggested the role of m^{6}A modification in cell fate determination and lineage transition in ESCs (Wang et al., 2014b; Geula et al., 2015; Zhao and He, 2015), prompting us to explore the potential function of m^{6}A modification in HSPC specification *in vivo*. To this end, we first profiled the m^{6}A methylome using MeRIP-seq in zebrafish embryos. Similar to that in mammals, the consensus RGACH motif enriched within m^{6}A peaks and the preferential distribution of m^{6}A peaks in the vicinity of the stop codon are also found in zebrafish, indicating that m^{6}A plays a conserved role across vertebrates. In order to investigate the function of m^{6}A modification, we next knocked-down the key m^{6}A methyltransferase coding gene *mettl3*, and observed a significantly-decreased m^{6}A level at global level. The GO analysis of MeRIP-seq data reveals that mRNAs with decreased m^{6}A enrichment upon *mettl3* knockdown are involved in embryonic development. Since *mettl3* expression is enriched in the endothelial and transitioning hemogenic endothelial cells, we then tried to uncover its possible function in developmental hematopoiesis. We observed the enhanced endothelial programming along with the failure of HSPC generation during EHT in *mettl3*-deficient embryos, demonstrating that m^{6}A modification written by METTL3 acts as a rheostat to repress the critical arterial endothelial genes, and instead, promote the hematopoietic specification (Zhang et al., 2017).

Mechanistically, we focused on the m^{6}A reader YTHDF2, which mediates the m^{6}A-modified mRNA decay (Wang et al., 2014a). Knockdown of *ythdf2* phenocopied the HSPC defects in *mettl3*-deficient embryos. Combinatorial analysis of YTHDF2-RIP-seq and MeRIP-seq uncovered *notch1a* mRNA, a critical regulator during EHT, as a key target. Further experiments revealed that the m^{6}A enrichment on *notch1a* mRNA was significantly decreased in *mettl3*-deficient embryos, while the mRNA expression of *notch1a* in endothelial cells of *mettl3*- or *ythdf2*-deficient embryos was significantly increased, confirming the hypothesis that depletion of *mettl3* enhances the endothelial programming through the delayed YTHDF2-mediated *notch1a* mRNA decay, thereby blocking the transition to HSPCs during EHT. We also identified the functional m^{6}A modification site on *notch1a* mRNA by miCLIP-seq, which contributes to the recognition of YTHDF2 for mRNA decay. This regulatory mechanism was also confirmed in mouse embryos, indicating the evolutionally conserved function of m^{6}A in HSC development in vertebrates.

Collectively, our findings reveal the hitherto unexplored distribution and function of mRNA m^{6}A methylation during vertebrate development and uncover the molecular mechanism underlying m^{6}A-dependent regulation in HSPC fate determination. This work also provides a new direction for the *in vitro* generation of HSCs.

Highlights in this study: (i) Distribution and characterization of m^{6}A mRNA modification is conserved in zebrafish embryos; (ii) METTL3-mediated m^{6}A mRNA modification controls the cell fate determination during EHT; (iii) YTHDF2-dependent *notch1* mRNA decay is required for HSPC specification; (iv) The function of m^{6}A in hematopoietic development is evolutionarily conserved.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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