

COMMENT

Open Access



Emerging links between m⁶A and misregulated mRNA methylation in cancer

Samie R. Jaffrey^{1*} and Michael G. Kharas^{2*}

Editorial summary

N⁶-methyladenosine (m⁶A) in mRNA has emerged as a crucial epitranscriptomic modification that controls cellular differentiation and pluripotency. Recent studies are pointing to a role for the RNA methylation program in cancer self-renewal and cell fate, making this a new and promising therapeutic avenue for investigation.

m⁶A, an epitranscriptomic mark that influences cellular differentiation

One of the hallmark features of cancer is misregulated gene expression. A newly recognized concept in the regulation of gene expression is that mRNAs contain a diverse set of modified nucleotides, and the location and identity of these modifications within the transcriptome constitute an 'epitranscriptomic' code. The initial concept of the epitranscriptome was introduced as a result of transcriptome-wide mapping of N⁶-methyladenosine (m⁶A), which revealed that m⁶A is found in at least a quarter of all mRNAs, typically near stop codons [1].

RNA methylation is mediated by a multiprotein 'writer' complex comprising RBM15–WTAP–METTL3–METTL14 [2]. METTL3 is the sole methyltransferase responsible for forming m⁶A, whereas RBM15 couples the methylation complex to mRNA to methylate adjacent m⁶A residues [2]. WTAP acts as an adaptor, coupling RBM15 to METTL3, whereas METTL14 positions RNA substrates for methylation by METTL3. Notably, adenosine methylation is reversible. Although FTO (fat mass and obesity-associated protein) was reported to be an m⁶A demethylase, it is now known that AlkB family member 5 (ALKBH5) is the only enzyme to show physiologically relevant demethylation activity in vivo [3].

Under normal conditions, the most prominent effect of the presence of m⁶A is to induce mRNA degradation, but, in response to certain types of cellular stress, the m⁶A distribution across the transcriptome can change, with the most notable effect being increases in the abundance of m⁶A marks in the 5'-untranslated region of select mRNAs [4]. This methylation confers to the mRNA the ability to be translated in a manner that does not require the canonical cap-binding protein eIF4E [4]. eIF4E-independent translation is activated in diverse disease states, especially cancer.

A connection between m⁶A and cancer-relevant processes is suggested from studies linking m⁶A to differentiation pathways that control stem cell fate [5]. Pluripotent stem cells depleted of m⁶A show marked resistance to stimuli that promote differentiation. These cells retain pluripotency markers and fail to acquire gene expression patterns seen in differentiated cells. By contrast, primed stem cells, which lack the ability to contribute to blastocyst chimeras, are more prone to differentiate and show enhanced and abnormal expression of differentiation markers upon depletion of m⁶A [5]. These studies show that alterations in m⁶A levels can alter differentiation pathways. As the pathways involved in embryonic stem cell maintenance and differentiation have been directly linked to the acquisition of stem cell properties in both solid and hematological malignancies, m⁶A alterations might have a role in cancer development (Fig. 1). Hypoxic environments and dysregulation of hypoxia-inducible factors (HIFs) have been implicated in a variety of cancers, including brain, lung, pancreatic, colon, ovarian, and many other cancers.

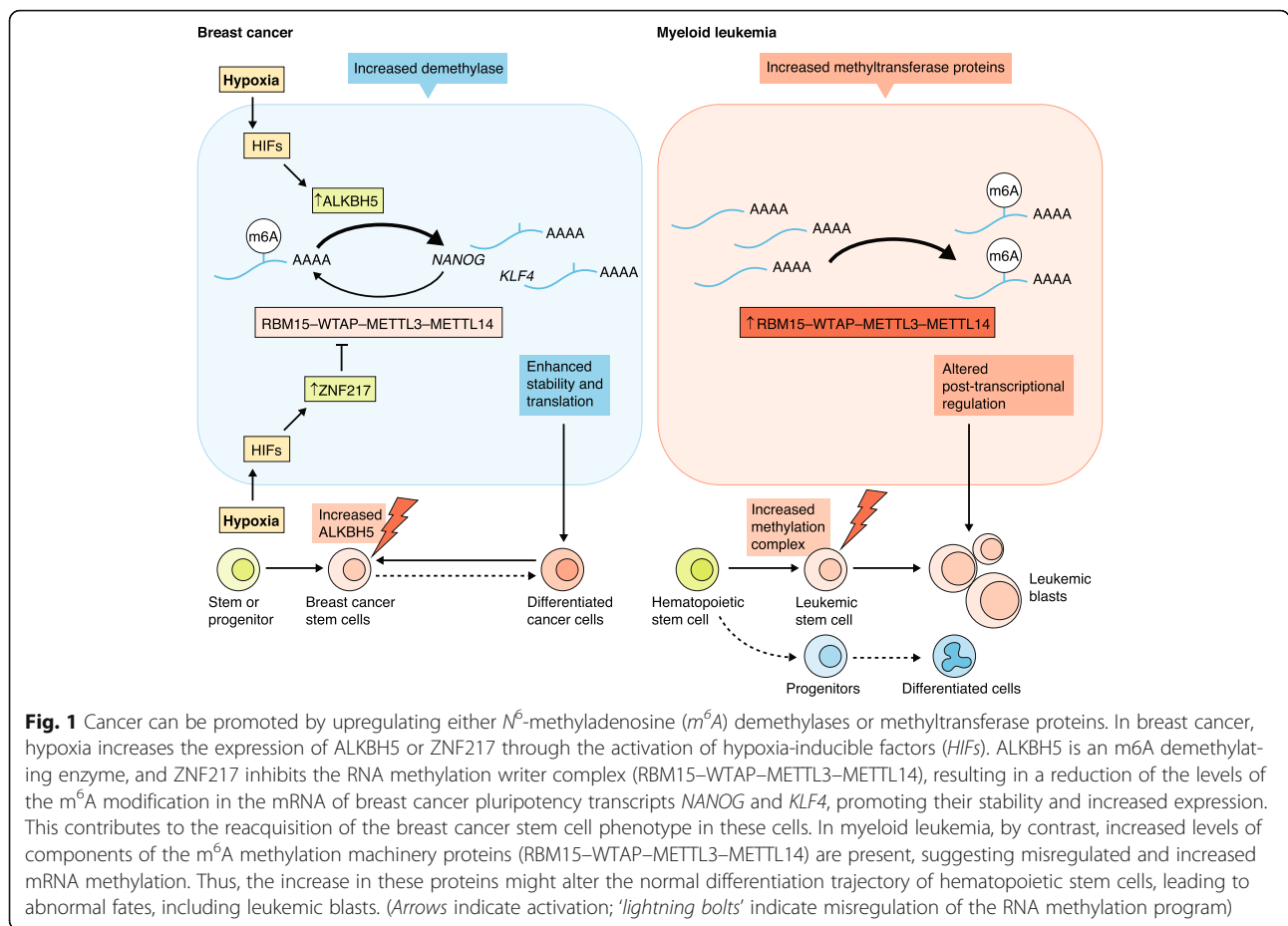
ALKBH5 and m⁶A depletion as a driver of cancer stem cell formation

In line with the above, recent studies point to a link between alterations in m⁶A levels and the abnormal cellular differentiation states present in cancer. In a variety of tumors, cancer stem cell populations are readily detected in hypoxic niches. Semenza and colleagues showed that

* Correspondence: srij2003@med.cornell.edu; kharasm@mskcc.org

¹Department of Pharmacology, Weill Cornell Medical College, Cornell University, New York, NY 10065, USA

²Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA



hypoxia was associated with increased breast cancer stem cell formation and elevated levels of ALKBH5 in breast cancer [6]. Notably, ALKBH5 was required for acquisition of the breast cancer stem cell phenotype. The authors observed that hypoxia increased the stability of *NANOG* mRNA and its protein levels. This could reflect ALKBH5-mediated *NANOG* mRNA demethylation or an indirect effect of ALKBH5 expression on *m*⁶A levels in *NANOG* mRNA [6]. *NANOG* is a key transcription factor that is associated with pluripotency. In addition to the effects of hypoxia on *NANOG* mRNA, hypoxia induces the expression of the zinc-finger protein ZNF217, which inhibits nuclear methylation [6]. Induction of ZNF217 also reduces *m*⁶A levels in *NANOG*, as well as in *KLF4* [7]. The *KLF4* transcription factor is a pluripotency factor required for the maintenance of breast cancer stem cells. Thus, hypoxia reduces *m*⁶A levels to promote the formation of breast cancer cells (Fig. 1).

***m*⁶A elevations in hematologic malignancies**

While hypoxia decreases *m*⁶A levels, increases in the abundance of *m*⁶A might also predispose to cancer. This is supported by evidence that genes that encode proteins that contribute to the RNA methylation complex are

upregulated in myeloid leukemia (Fig. 1). Analysis of The Cancer Genome Atlas (TCGA; <https://cancergenome.nih.gov/>) shows that METTL3, METTL14, and RBM15 are highly expressed in myeloid leukemia compared with other cancers. These proteins appear to be required for maintaining the abnormal differentiation state seen in myeloid leukemia. A role for *m*⁶A in myeloid leukemias is supported by studies of WTAP depletion. Bansal and colleagues found that WTAP expression was elevated in cells derived from 32% of patients with acute myeloid leukemia [8]. WTAP knockdown results in reduced proliferation, increased differentiation, and increased apoptosis in a leukemia cell line [8]. WTAP knockdown is a highly efficient approach to deplete *m*⁶A from mRNA. Thus, *m*⁶A depletion might account for the anti-leukemia effects observed upon WTAP depletion.

RBM15, another component of the *m*⁶A writer complex, is also linked to myeloid leukemia. In this case, RBM15 has a clear driver role in the development of hematologic malignancy. Acute megakaryoblastic leukemias were shown to be mediated by a chromosomal translocation t(1;22) of *RBM15* (also called *OTT1*) with the *MAL* gene [9]. RBM15 has crucial roles in

maintaining quiescence in hematopoietic stem cells and in megakaryocyte leukemia cell line differentiation by controlling the splicing of key differentiation genes, including *GATA1*, *RUNX1*, *TALI*, and *c-MPL* [10]. Because RBM15 directs m⁶A formation in the transcriptome [2], the oncogenic effects of RBM15 overexpression and *RBM15-MAL* translocation might reflect aberrant m⁶A formation.

Although each of the major proteins in the m⁶A methylation complex—that is, RBM15, WTAP, METTL3, and METTL14—show alterations in myeloid leukemias, definitive demonstration of the role of m⁶A will require mechanistic evidence linking m⁶A alterations to leukemia phenotypes in these cancers.

Conclusions

The modification m⁶A is an epitranscriptomic mark that influences a wide variety of RNA processing steps, including splicing, mRNA stability, and translation. Genes associated with pluripotency and lineage-specific differentiation are controlled by m⁶A levels, and reduced m⁶A levels can lead to a misregulation of these genes and the acquisition of stem cell characteristics. Alternatively, increases in m⁶A levels are expected to stabilize these transcripts and would therefore be particularly problematic in tissues that are continuously replenished from a stem cell population, such as the hematopoietic lineage. Hematopoietic stem cells traverse through distinct differentiation intermediates in order to achieve their final differentiated state. Elevations in m⁶A might alter the normal differentiation pathway, resulting in cells being trapped in a progenitor cell state.

Many unanswered questions remain. How conserved are these pathways in other cancer types? Many cancer subtypes are associated with abnormal differentiation states or cancer stem cells, making it likely that interventions that influence m⁶A levels could therapeutically alter the differentiation program. Will a systematic analysis of the marked transcripts in cancer reveal new targets for therapeutic intervention? Can pharmacologic modulation of the RNA methylation program in various cancers push cells toward differentiation? Another important question is whether targeting m⁶A would have unwanted side-effects. As m⁶A might be used in every cell for the regulation of gene expression, targeting m⁶A might not provide a suitable therapeutic index. Finally, the high reliance of myeloid leukemia cells on methylation complex proteins raises the hope that these cells will show higher sensitivity to m⁶A pathway inhibitors than other cell types.

Abbreviations

m⁶A: N⁶-methyladenosine; TCGA: The Cancer Genome Atlas

Authors' contributions

SRJ and MGK drafted the manuscript, and both authors approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Published online: 12 January 2017

References

- Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell*. 2012;149:1635–46.
- Patil DP, Chen CK, Pickering BF, Chow A, Jackson C, Guttman M, et al. m6A RNA methylation promotes XIST-mediated transcriptional repression. *Nature*. 2016;537:369–73.
- Mauer J, Luo X, Blanjoie A, Jiao X, Grozhik AV, Patil DP, et al. Reversible methylation of m6Am in the 5' cap controls mRNA stability. *Nature*. 2016. doi: 10.1038/nature21022
- Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, et al. 5' m(6)A promotes cap-independent translation. *Cell*. 2015;163:999–1010.
- Geula S, Moshitch-Moshkovitz S, Dominissini D, Mansour AA, Kol N, Salmon-Divon M, et al. Stem cells. m6A mRNA methylation facilitates resolution of naive pluripotency toward differentiation. *Science*. 2015;347:1002–6.
- Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, et al. Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m(6)A-demethylation of NANOG mRNA. *Proc Natl Acad Sci U S A*. 2016;113:E2047–56.
- Zhang C, Zhi W, Lu H, Samanta D, Chen I, Gabrielson E, et al. Hypoxia-inducible factors regulate pluripotency factor expression by ZNF217- and ALKBH5-mediated modulation of RNA methylation in breast cancer cells. *Oncotarget*. 2016. doi: 10.18632/oncotarget.11743.
- Bansal H, Yihua Q, Iyer SP, Ganapathy S, Proia DA, Penalva LO, et al. WTAP is a novel oncogenic protein in acute myeloid leukemia. *Leukemia*. 2014;28:1171–4.
- Mercher T, Coniat MB, Monni R, Mauchauffe M, Nguyen Khac F, Gressin L, et al. Involvement of a human gene related to the *Drosophila* spen gene in the recurrent t(1;22) translocation of acute megakaryocytic leukemia. *Proc Natl Acad Sci U S A*. 2001;98:5776–9.
- Zhang L, Tran NT, Su H, Wang R, Lu Y, Tang H, et al. Cross-talk between PRMT1-mediated methylation and ubiquitylation on RBM15 controls RNA splicing. *Elife*. 2015;4:e07938.