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Role of N^6-methyladenosine modification in cancer

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As the most abundant internal modification in eukaryotic messenger RNAs identified, N^6-methyladenosine (m^6A) has been shown recently to play essential roles in various normal bioprocesses. Evidence is emerging that m^6A modification and its regulatory proteins also play critical roles in various cancers including leukemia, brain tumor, breast cancer and lung cancer, etc. For instance, FTO, the first m^6A demethylase identified, has been reported recently to play an oncogenic role in leukemia and glioblastoma. ALKBH5 (another m^6A demethylase) has been reported to exert a tumor-promoting function in glioblastoma and breast cancer. METTL3 (a major m^6A methyltransferase) likely plays distinct roles between glioblastoma and lung cancer. Here we discuss the recent progress and future prospects in study of m^6A machinery in cancer.

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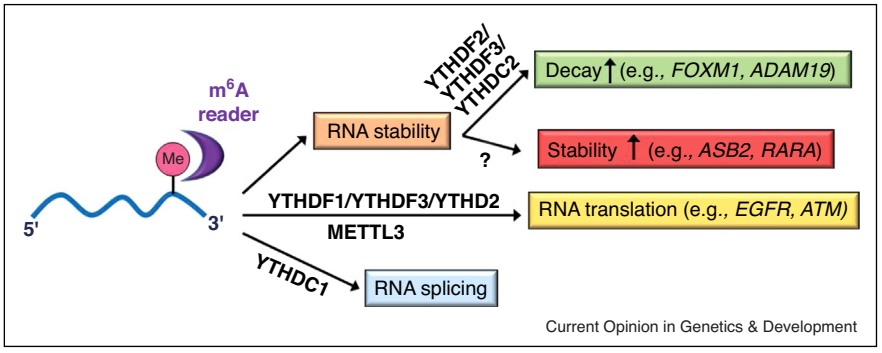
Introduction

N^6-methyladenosine (m^6A) is the most abundant internal modification in eukaryotic messenger RNAs (mRNAs) that mainly occur at consensus motif of RRm^6ACH ([G/A/U][G > A]m^6AC[U > A > C] [1,2]. Although m^6A was first discovered in 1970s [3,4], functional characteristics and regulatory mechanisms of m^6A modification were largely unknown until recent years [1,2]. The identification of the fat mass and obesity-associated protein (FTO) as a *bona fide* demethylase of m^6A modification [5] and the development of transcriptome-wide approaches for m^6A sequencing [6,7] have indicated that m^6A is a reversible and dynamic RNA modification that may affect thousands of mRNAs and non-coding RNAs in a given type of cells. The deposition of m^6A is catalyzed by the m^6A methyltransferase complex (MTC) composed of methyltransferase-like 3 and 14 (METTL3 and METTL14) (i.e., writers) and their cofactor, Wilms tumor 1-associated protein (WTAP) [8–11]. The removal of m^6A is facilitated by FTO and ALKBH5, two m^6A demethylase (i.e., erasers) that may target distinct sets of target mRNAs [2,5,12]. YTHDF1, YTHDF2, YTHDF3, and YTHDC1, members of the YT521-B homology (YTH) domain family of proteins, have been identified as m^6A direct readers that affect the translation, stability, and/or splicing of target mRNAs [13–17] (see Figure 1). Recent studies have shown that m^6A modification in mRNAs or non-coding RNAs plays essential roles in virtually all types of normal bioprocesses including tissue development, self-renewal and differentiation of stem cells, heat shock response, circadian clock control, DNA damage response, and maternal-to-zygotic transition, likely through affecting RNA fate/metabolism and functions such as mRNA stability, splicing, transport, localization, translation, primary microRNA processing, and RNA–protein interactions [6,7,10,12–14,18–26]. While still in the beginning stage, efforts have also been made to investigate the biological impacts of m^6A modification in cancer. In this review, we summarize the recent advance in our understanding of the biological functions and underlying molecule mechanisms of m^6A regulatory proteins (i.e., writers, erasers and readers) in various types of cancers, and also discuss future prospects.

FTO plays an oncogenic role in leukemia as an m^6A demethylase

FTO was first reported to be associated with increased body mass and obesity in humans [27–30]. In line with a link between the single nucleotide polymorphism (SNP) risk genotype and increased FTO expression in human blood cells and fibroblasts [31,32], transgenic mouse model studies have demonstrated a critical role of FTO in regulating fat mass, adipogenesis and body weight [33,34,35•], though IRX3 has also been suggested to be associated with obesity-associated variants within *FTO* [36]. The identification of FTO as the first m^6A demethylase suggests that FTO involves in m^6A-based post-transcriptional regulation of RNA targets. Through analysis of genome-wide gene expression profiles of several large-cohorts of human primary acute myeloid

Figure 1



The fates of m^{6}A-modified mRNA transcripts are influenced by different m^{6}A readers. See Refs. [13–17] for more details. The examples of m^{6}A-modification affected target mRNAs shown herein are those that have been reported to be dysregulated in cancer (see Figure 2 and Table 1 for more information). While many m^{6}A-modified mRNA transcripts (e.g., *FOXM1* and *ADAM19*) can be recognized by readers such as YTHDF2, YTHDF3 and/or YTHDC2 that promote mRNA decay, other m^{6}A-modified mRNA transcripts (e.g., *ASB2* and *RARA*) can be recognized by some currently unknown m^{6}A readers that promote mRNA stability. Different m^{6}A readers can also promote translation or affect splicing of m^{6}A-modified target mRNAs. Besides serving as an m^{6}A methyltransferase in nucleus, METTL3 may also serve as an m^{6}A reader in cytoplasm in some scenarios (e.g., Ref. [51••]).

leukemia (AML) patients, Li *et al.* found that *FTO* is highly expressed in certain subtypes of AMLs including AMLs carrying t(11q23)/MLL-rearrangements, t(15;17)/PML-RARA, NPM1 mutation (i.e., cytoplasmic localization of NPM1 (NPM1c+)), and/or Fms-like tyrosine kinase 3 with internal tandem duplication (FLT3-ITD) [37••]. More importantly, they provided compelling evidence, based on both *in vitro* leukemia cell line models and *in vivo* mouse leukemia models, showing that FTO plays an essential oncogenic role in promoting leukemic cell transformation and AML cell survival/growth and enhancing leukemogenesis, as well as in inhibiting all-trans-retionic acid (ATRA)-induced differentiation of AML cells [37••] (see Figure 2, upper left; Table 1).

Mechanistically, FTO functions as an m^{6}A demethylase that post-transcriptionally regulates expression of its critical target RNAs (such as *ASB2* and *RARA*) in an m^{6}A-dependent manner. *ASB2* and *RARA* have been implicated in leukemia cell growth and drug response, especially in ATRA-induced AML cell differentiation [38–40]. FTO negatively regulates expression of *ASB2* and *RARA* through reducing the m^{6}A abundance of the target RNA transcripts (especially in the 3′ untranslated regions (3′-UTRs)) and thereby decreasing the stability of the RNA transcripts [37••] (see Figure 2, upper left). Notably, in this study, the luciferase-reporter and mutagenesis assays have been introduced into the field of m^{6}A-related research, for the first time, to demonstrate that the putative m^{6}A consensus motif sites on the target RNA transcripts are important for the m^{6}A-based epigenetic regulation of the target mRNA transcripts [37••]. A recent study suggests that FTO also exhibits demethylation activity towards RNA with N^6,2′-*O*-dimethyladenosine (m^{6}A$_m$) in the 5′ cap, as a subform of m^{6}A, leading to enhanced mRNA stability [41]. Nonetheless, roughly $^1/_4$ of FTO target mRNAs may contain an A as the first encoded nucleotide adjacent to the 7-methylguanosine (m^{7}G) cap and there is a pretty low chance that this A is methylated at both N^6 and 2′-O sites; in contrast, on average there are 3–5 internal m^{6}A sites per mRNA transcript [42]. Thus, the overall abundance of internal m^{6}A modifications should be much higher than that of the 5′ cap m^{6}A$_m$ modification in cells. Indeed, analysis of the m^{6}A-seq data reported in [37••] showed that over 95% of the m^{6}A peaks with increased abundance upon *FTO* knockdown in AML cells are located in the internal regions (>150 nucleotides away from the 5′ ends) and thus are impossible 5′ cap m^{6}A$_m$. In addition, liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based quantification of m^{6}A and m^{6}A$_m$ in human AML cells showed that the internal m^{6}A abundance is approximately 20–30 times of the near 5′ cap m^{6}A$_m$ abundance, and the internal m^{6}A peaks are the main substrates of FTO and represent the major changes in the N^6-methyladenosine abundance when *FTO* is forced expressed or knocked down in AML cells (Su R, *et al.*, unpublished). Moreover, as demonstrated by the luciferase reporter and mutagenesis assays, the internal m^{6}A sites on *ASB2* and *RARA* transcripts are required for FTO-mediated post-transcriptional regulation of their stability and expression [37••]. Therefore, internal m^{6}A sites, rather than the rare m^{6}A$_m$ in the 5′ cap, are responsible for FTO-mediated post-transcription regulation of target mRNAs in cancer cells.

Dysregulation of other m^{6}A regulatory proteins in leukemia

WTAP, which was first identified as a partner of Wilms' tumor gene 1 (WT1), has been reported recently to play