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Review

Refining the role of N^6-methyladenosine in cancer

Jonas Koch and Frank Lyko

N^6-methyladenosine (m^{6}A) is the most abundant internal modification of eukaryotic mRNAs. m^{6}A affects the fate of its targets in all aspects of the mRNA life cycle and has important roles in various physiological and pathophysiological processes. Aberrant m^{6}A patterns have been observed in numerous cancers and appear closely linked to oncogenic phenotypes. However, most studies relied on antibody-dependent modification detection, which is known to suffer from important limitations. Novel, antibody-independent, quantitative approaches will be critical to investigate changes in the m^{6}A landscape of cancers. Furthermore, pharmaceutical targeting of the m^{6}A writer Methyltransferase-like 3 (METTL3) has demonstrated the potential to modulate cancer cell phenotypes. However, the enzyme also appears to be essential for the viability of healthy cells. Further refinement of therapeutic strategies is therefore needed to fully realize the potential of m^{6}A-related cancer therapies.

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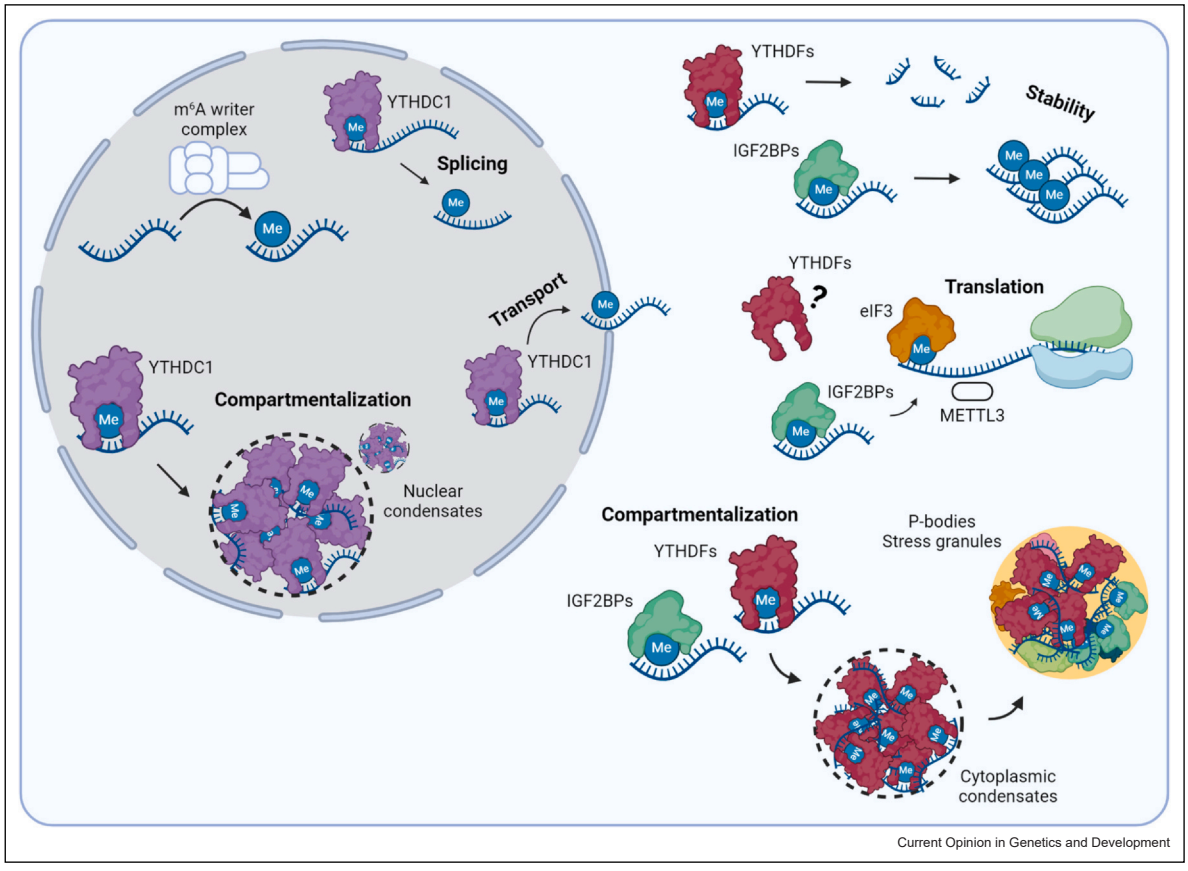
N^6-methyladenosine (m^{6}A) is the most investigated internal modification of eukaryotic mRNAs. It is catalyzed by a multiprotein complex, with Methyltransferase-like 3 (METTL3) being the catalytically active writer component. The modification has important roles in physiological and pathophysiological processes, including cellular differentiation and cancer development. Cancer-related changes of m^{6}A and their potential role(s) in tumorigenesis have been described elsewhere [1–4]. In this mini-review, we focus on recent developments in the characterization of molecular mechanisms of m^{6}A-dependent transcript regulation, particularly the m^{6}A-dependent compartmentalization of mRNAs into phase-separated condensates. We also highlight the technical challenges of measuring cancer-associated changes in m^{6}A patterns and discuss novel developments that might enable the quantitative mapping of m^{6}A transcriptomes at single-base resolution. Finally, we provide an updated assessment of the role of METTL3 as an oncogene or tumor suppressor in different cancer entities. We discuss novel developments in the use of METTL3 inhibitors for cancer therapy, with a particular focus on the therapeutic potential of m^{6}A-dependent condensates and METTL3 inhibition as an emerging strategy in cancer immunotherapy.

Molecular mechanisms of N^6-methyladenosine-dependent transcript regulation

m^{6}A has been shown to affect the fate of its target transcripts in multiple ways, including alternative splicing, nuclear transport, stability, and translation (Figure 1). These m^{6}A-dependent functions are usually translated by m^{6}A reader proteins that directly or indirectly bind to m^{6}A sites [5]. Reader proteins of the YTH family essentially comprise the nuclear YTHDC1 reader and the cytoplasmic YTHDF1–3 readers that directly bind to m^{6}A sites via their YTH domain. While earlier studies reported distinct roles for distinct YTHDF proteins, a more recent model described all cytoplasmic readers to have redundant binding sites and to function essentially in mRNA decay by networking with components that are linked to the Carbon catabolite repression 4-negative on TATA-less (CCR4-NOT) degradation complex [5–7]. In contrast, the nuclear YTHDC1 reader was shown to affect essential steps in pre-mRNA processing, including alternative splicing and nuclear export of transcripts [5,7]. Interestingly, insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs) were also shown to bind m^{6}A-modified transcripts, most likely via their KH domain [8]. Together with the co-factors HuR and MATR3, IGF2BPs can promote the stabilization of their target transcripts, thereby facilitating their translation [8,9]. Since m^{6}A also affects the secondary structures and the accessibility of its transcripts, other nondirect m^{6}A reader proteins were described to mediate m^{6}A-related functions [6]. For example, heterogeneous nuclear ribonucleoproteins (hnRNPs) hnRNPC and hnRNPG belong to this class of indirect reader proteins.

Figure 1



Molecular mechanisms of m^{6}A-mediated mRNA regulation. The fate of m^{6}A-modified mRNAs is regulated by m^{6}A readers essentially comprising the nuclear YTHDC1 protein and the cytosolic YTHDF proteins. YTHDC1 was shown to control splicing and nuclear export of mRNAs. Furthermore, the reader was found to promote the recruitment of mRNAs into nuclear YTHDC1-m^{6}A condensates, thereby affecting the stability of the transcripts. The most current model for cytoplasmic YTHDF proteins proposes an essential role in mRNA decay, while their role in translation needs further validation. Also, YTHDF proteins were shown to undergo phase separation and to further fuse with cytoplasmic condensates, including stress granules and P-bodies. Thereby, they promote the phase separation potential of bound transcripts and affect their localization and regulation. Mechanisms by which m^{6}A-modified mRNAs are regulated by IGF2BPs comprise stabilization, translation, as well as compartmentalization. Created with BioRender.com.

Additional insight about the molecular function of YTHDF proteins came from a combination of structural and cell biological analyses. YTHDF1–3 are paralogs that share high sequence similarity. Besides the C-terminal YTH domain, they also possess low complexity and intrinsically disordered regions [10]. It was shown that upon interaction of multiple readers with poly-m^{6}A-modified transcripts, the readers were scaffolded and stabilized via their low complexity regions, which promoted liquid–liquid phase separation into condensates. These dynamic m^{6}A-mRNA-reader condensates could further separate into P-bodies or stress granules, thereby adding a novel layer of m^{6}A-dependent transcript regulation [11] (Figure 1). Additional findings showed that the depletion of YTHDF1 and YTHDF3 inhibits stress granule formation [12] and that m^{6}A mediates the length-dependent enrichment of transcripts in stress granules [13]. Furthermore, the IGF2BP readers were found to affect transcript regulation via their translocation into P-bodies and stress granules, thereby promoting their stabilization [9] (Figure 1). Most recently, the IGF2BP3 reader was found to regulate the partitioning of m^{6}A-marked transcripts into actively translating and nontranslating pools. It was shown that polysome-associated transcripts are generally low in m^{6}A sites, while P-body-associated transcripts are hyper-m^{6}A-methylated. Global reduction of m^{6}A in HeLa cells caused a translocation of mRNAs from P-bodies to polysomes [14].

Similar to YTHDF proteins, YTHDC1 also possesses an internal YTH domain and intrinsically disordered regions that were shown to promote the formation of nuclear YTHDC1-m^{6}A condensates via phase separation (Figure 1). In acute myeloid leukemia (AML) cells, YTHDC1 was found to recruit oncogenic MYC transcripts into

condensates, thereby protecting them from degradation via the PAXT-exosome complex and promoting AML cell survival and differentiation suppression [15]. It was also shown that the recognition of m^{6}A-modified MALAT1 transcripts affects the composition of nuclear speckles, thereby regulating gene expression in esophageal cancer cells [16]. Taken together, these studies illustrate how phase separation and regulation via compartmentalization into nuclear and cytoplasmic condensates affect the fate of poly-m^{6}A-marked transcripts.

Finally, while most m^{6}A-related effects on transcript regulation appear to be mediated by m^{6}A reader proteins, it was also shown that m^{6}A can directly promote cap-independent translation of mRNAs via different mechanisms (Figure 1). eIF3 facilitates cap-independent translation by directly binding to m^{6}A in the 5′UTR of transcripts, while METTL3 promotes mRNA circularization to initiate translation [6,17].

Challenges in the detection of cancer-associated N^6-methyladenosine changes

To date, little is known about cancer-associated changes in m^{6}A levels and distribution. A major problem for the robust detection of these differences is the quantification of m^{6}A in the transcriptome. Many studies have used dot blot and Enzyme-linked immunosorbent assays (ELISA) for the global quantification of m^{6}A levels in cell lines and patient samples. However, these techniques rely on m^{6}A antibodies that are known to be cross-reactive [18,19]. Furthermore, purification of mRNA from total RNA samples is challenging as contaminating rRNA species, that also contain m^{6}A and make up more than 90% of the cellular RNA, can be difficult to deplete [20].

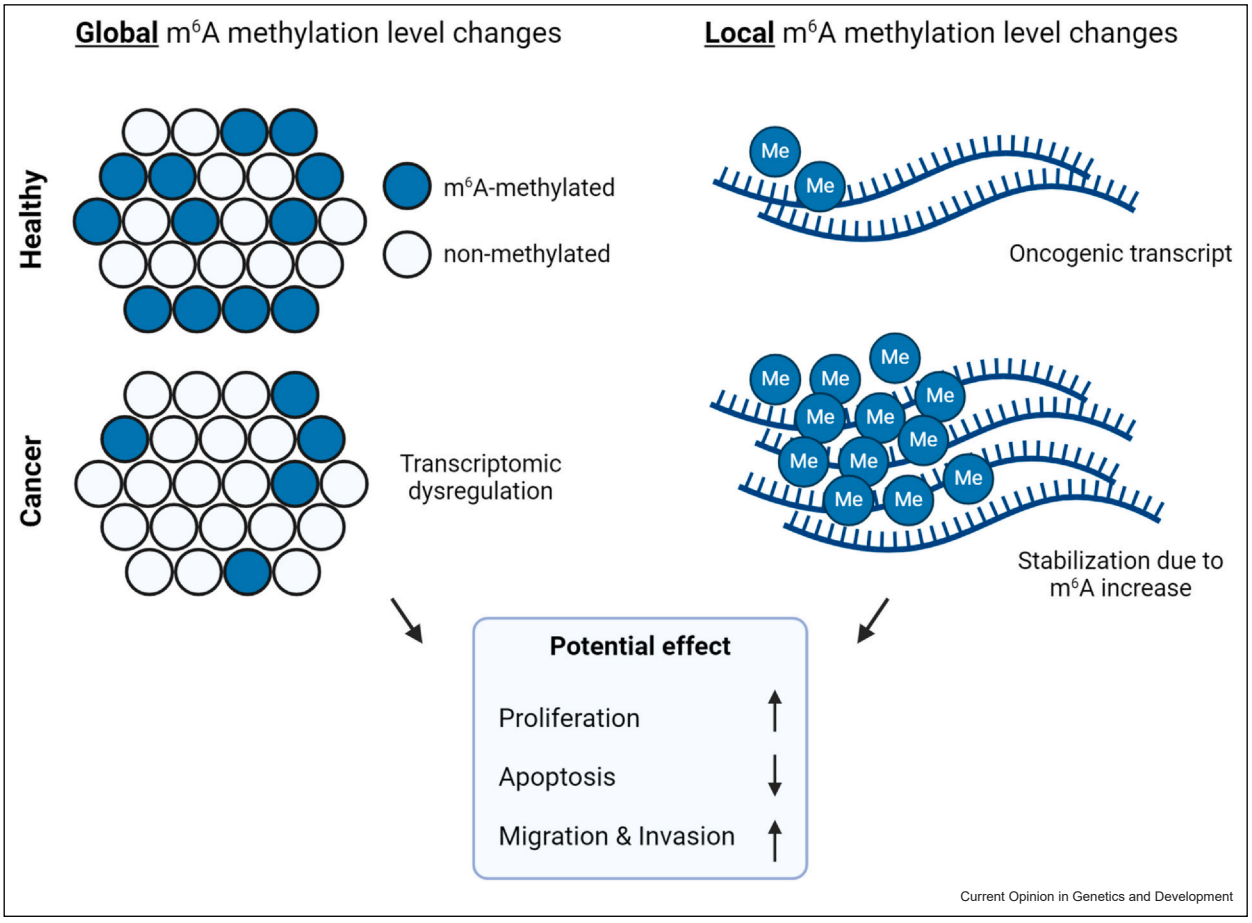
Liquid chromatography–tandem mass spectrometry (LC-MS/MS) allows for a more rigorous quantification of global m^{6}A levels. By the use of external calibrations, spike-in measurements, and radio-labeled internal standards, LC-MS/MS achieves high sensitivity and measures RNA modifications within the femtomole range [21]. The parallel detection of rRNA-specific modifications (e.g. $m_2{}^6A$) allows for an estimation of contaminating rRNA amounts between different mRNA sample preparations. Thus, measured differences in m^{6}A levels between mRNA samples are reliable, and most likely not the result of different levels of contaminating rRNA. Using this approach, we have recently shown a pronounced global reduction of m^{6}A methylation levels in bladder cancer tissues compared with para-tumoral tissues (n = 20) [22]. On average, m^{6}A levels were decreased by nearly 50% in tumor samples. Expression analyses of the m^{6}A writer complex revealed that METTL3 was upregulated, while several subunits were downregulated in the tumor samples, suggesting that a stochiometric imbalance within the writer complex might cause the observed global m^{6}A decrease [22].

On the molecular level, a global reduction of m^{6}A likely affects transcript-specific m^{6}A signatures that impact the fate of a cell (Figure 2). For investigating the differences in the transcriptome-wide distribution of m^{6}A sites, quantitative m^{6}A mapping approaches need to be pursued. Again, most studies rely on antibody-dependent mapping techniques for the detection of m^{6}A sites. The immunoprecipitation of RNA using m^{6}A-recognizing antibodies allows for an enrichment of modified mRNAs that are sequenced, before modification peaks are called. However, these methods also suffer from limited antibody specificity that include cross-reactivity with other RNA modifications, and from poor resolution, since m^{6}A modification sites can only be localized to regions spanning 200–300 nucleotides [18,19]. To counteract these disadvantages, antibody-independent mapping techniques are being established. Nanopore sequencing enables direct RNA sequencing by pulling RNA molecules through a membrane-embedded protein pore. Changes in the constantly measured current intensity result in specific profiles that allow for the computational identification of the respective nucleotide, while the molecule passes the pore. Modified RNA nucleobases such as m^{6}A have characteristic current intensity profiles, which allows their direct detection [23,24]. Another emerging method is GLORI, which allows unbiased, antibody-independent, base-resolution, and transcriptome-wide m^{6}A mapping on standard next-generation sequencing platforms [25]. The method uses glyoxal and sodium nitrite for the deamination of unmodified adenosines into inosines under conditions where m^{6}A remains resistant to deamination. With this approach, more than 170 000 m^{6}A sites with a median methylation level of approximately 40% were identified in the transcriptome of the human HEK293T cell line. One-third of the detected m^{6}A sites appeared in clusters that were characterized by higher methylation levels and important for gene regulatory functions [25]. Thus, GLORI has the potential to become the gold standard for quantitative profiling of m^{6}A in the transcriptome. Comparative modification mapping of mRNAs from tumor and matched control samples should be used to establish the epitranscriptomic landscape of human cancers and to define m^{6}A-dependent, cancer-associated pathways.

N^6-methyladenosine/METTL3 as a therapeutic target

In recent years, numerous studies have investigated the role of METTL3 in various cancer entities (Table 1). For most tumors, METTL3 was found to have tumor-promoting functions. However, the protein was also shown to have tumor-suppressive roles in endometrial and kidney cancers. In bladder, breast, colorectal, and thyroid cancers, both tumor-suppressive and oncogenic functions of METTL3 were reported.

Figure 2



Cancer-associated changes of m^{6}A. Numerous studies have reported global changes of m^{6}A methylation levels and patterns in different tumor entities, potentially causing a transcriptomic dysregulation that promotes oncogenic phenotypes. Prominent examples include the global reduction of m^{6}A in bladder cancer [22] (left panel) and the increased m^{6}A modification levels of the ZMYM1 mRNA that enhanced its stability, thereby promoting gastric cancer metastasis [64] (right panel). Created with BioRender.com.

METTL3 has been proposed as a potential drug target due to its described oncogenic roles in most investigated tumors (Table 1). In AML, small-molecule-based inhibition of METTL3 via STM2457 led to a reduced oncogenic phenotype of AML cells. Mechanistically, the m^{6}A methylation levels of leukemogenic transcripts were reduced upon inhibitor treatment, thereby interfering with their expression and translation. *In vivo* METTL3 inhibition caused impaired engraftment and prolonged survival in a mouse leukemia model without significant side effects [26]. STM2457-based inhibition of METTL3 also reduced the oncogenic phenotype of small cell lung cancer cells and could reverse chemoresistance both *in vitro* and *in vivo* at a concentration that did not affect the proliferation of healthy lung epithelial cells [27]. Additional studies indicated therapeutic effects in a variety of solid tumor entities [28–31]. The molecular mode of action of METTL3 inhibition remains to be fully understood.

Considering the basic cellular functions of m^{6}A and the role of METTL3 as the predominant, if not only, m^{6}A mRNA methyltransferase in mammalian cells, it will be important to resolve whether METTL3 is a tumor-specific oncogene or whether it is more broadly required for cellular viability. METTL3 knockout mice completely lack m^{6}A methylation and fail to terminate naïve pluripotency, resulting in early embryonic lethality [32]. The recent identification of catalytically active METTL3 isoforms in CRISPR/Cas9 knockout cells further suggests that METTL3 is essential for the viability of cells [33]. This observation is also consistent with dependency analyses that characterize METTL3 as a pan-essential gene [22,33]. Pan-essential genes are often misidentified as tumor-selective genes due to limited preclinical modeling, and their drugs are often characterized by a low therapeutic index, where efficacy is limited by off-target toxicities as well as difficulties in patient stratification [34]. Strategies for increasing the therapeutic index for

Table 1

Functional role of METTL3 in selected tumors. Representative studies were cited.

Entity	Oncogene	Tumor suppressor	Reference
Acute myeloid leukemia	X		[44]
Cervical cancer	X		[45]
Gastric cancer	X		[46]
Head and neck cancer	X		[47]
Liver cancer	X		[48]
Lung cancer	X		[49]
Melanoma	X		[50]
Pancreatic cancer	X		[51]
Prostate cancer	X		[52]
Ovarian cancer	X		[53]
Bladder cancer	X	X	[54] (Oncogene), [55] (Suppressor)
Breast cancer	X	X	[56] (Oncogene), [57] (Suppressor)
Colorectal cancer	X	X	[58] (Oncogene), [59] (Suppressor)
Thyroid cancer	X	X	[60] (Oncogene), [61] (Suppressor)
Endometrial cancer		X	[62]
Kidney cancer		X	[63]

METTL3 inhibitors potentially include efforts that reduce their systemic exposure, such as tumor-selective drug delivery systems, local drug administration, or prodrug approaches. Challenges and opportunities related to the targeting of pan-essential genes or pathways have been reviewed elsewhere [34,35].

An alternative, potentially more tumor-selective strategy might be the targeting of m^{6}A-dependent, pathophysiological condensates. For example, the protection of m^{6}A-modified MYC transcripts via YTHDC1-mediated compartmentalization into nuclear condensates was shown to promote AML progression [15]. Thus, targeting of these condensates might have significant therapeutical potential. Small molecules or peptides, that inhibit the interaction between m^{6}A readers and their methylated targets, could prevent the initiation of oncogenic condensates or promote their dissociation. While it remains to be resolved to what extent phase-separated compartments are druggable, it has been shown that chemotherapeutics can selectively cluster in condensates [36]. Furthermore, screening platforms for the identification of so-called condensate modifying drugs (c-mods) have been established in order to restore physiological condensate states in diseases [37,38].

METTL3 inhibition in cancer immunotherapy

Most recently, the role of METTL3 in the tumor microenvironment (TME), immune escape mechanisms, and antitumor immunity has become a topic of high interest. When the novel METTL3 inhibitor STM3006 was used to decrease global m^{6}A methylation levels in a human ovarian and a mouse breast cancer cell line, it induced the formation of double-stranded RNA (dsRNA). dsRNA sensing and Retinoic acid-inducible gene 1 (RIG-I)-like receptor signaling triggered a cell-intrinsic interferon response that increased antitumor immunity [39]. In *in vitro* killing assays, METTL3 inhibition enhanced the potency of CD8+ T cells and led to increased tumor cell killing. Furthermore, METTL3 inhibition was found to be as efficacious as PD-1 checkpoint blockade, and the combinatory use of both treatments even outperformed the single agents in a range of immunocompetent mouse models comprising hematologic and solid cancers [39]. Thus, METTL3 inhibitors were suggested to augment antitumor immunity in cancer therapies.

In bladder cancer, RIG-I was identified as a downstream target of YTHDF2 [40]. YTHDF2 mediated the degradation of RIG-I transcripts, leading to immune evasion of bladder cancer cells. *In vivo*, YTHDF2-deficient bladder cancer cells were more susceptible to a stronger immune response and characterized by increased CD8+ T-cell infiltration, thereby also boosting the efficacy of Bacillus Calmette-Guerin immunotherapy [40]. These findings describe an oncogenic METTL3/YTHDF2/RIG-I axis that has a potential role in antitumor immunity.

Finally, METTL3 was also shown to promote non-alcoholic fatty liver disease–derived hepatocellular carcinoma by reducing tumor infiltration of CD8+ T-cells [41]. STM2457 and anti-PD-1 combination therapy restored CD8+ T-cell infiltration, thereby promoting tumor regression [41]. In colorectal and non–small-cell lung cancer, METTL3 mediated the formation of an immunosuppressive TME, characterized by an accumulation of myeloid-derived suppressor cells and decreased potency of CD4+ and CD8+ T-cells. METTL3 inhibition led to the reprogramming of the TME favoring the infiltration of potent immune cells and potentiated the effects of anti-PD-1 therapy [42,43]. Taken

together, these findings reinforce a role for METTL3 in anticancer immunity and refine the therapeutic potential of METTL3 inhibitors.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Deng X, Qing Y, Horne D, Huang H, Chen J: **The roles and implications of RNA m(6)A modification in cancer**. *Nat Rev Clin Oncol* 2023, **20**:507-526.
2. Barbieri I, Kouzarides T: **Role of RNA modifications in cancer**. *Nat Rev Cancer* 2020, **20**:303-322.
3. Vu LP, Cheng Y, Kharas MG: **The biology of m(6)A RNA methylation in normal and malignant hematopoiesis**. *Cancer Discov* 2019, **9**:25-33.
4. Primac I, Penning A, Fuks F: **Cancer epitranscriptomics in a nutshell**. *Curr Opin Genet Dev* 2022, **75**:101924.
5. Flamand MN, Tegowski M, Meyer KD: **The proteins of mRNA modification: writers, readers, and erasers**. *Annu Rev Biochem* 2023, **92**:145-173.
6. He PC, He C: **m(6) A RNA methylation: from mechanisms to therapeutic potential**. *Embo J* 2021, **40**:e105977.
7. Murakami S, Jaffrey SR: **Hidden codes in mRNA: control of gene expression by m(6)A**. *Mol Cell* 2022, **82**:2236-2251.
8. Duan M, Liu H, Xu S, Yang Z, Zhang F, Wang G, Wang Y, Zhao S, Jiang X: **IGF2BPs as novel m(6)A readers: diverse roles in regulating cancer cell biological functions, hypoxia adaptation, metabolism, and immunosuppressive tumor microenvironment**. *Genes Dis* 2024, **11**:890-920.
9. Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, Zhao BS, Mesquita A, Liu C, Yuan CL, *et al.*: **Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation**. *Nat Cell Biol* 2018, **20**:285-295.
10. Patil DP, Pickering BF, Jaffrey SR: **Reading m(6)A in the transcriptome: m(6)A-binding proteins**. *Trends Cell Biol* 2018, **28**:113-127.
11. Ries RJ, Zaccara S, Klein P, Olarerin-George A, Namkoong S, Pickering BF, Patil DP, Kwak H, Lee JH, Jaffrey SR: **m6A enhances the phase separation potential of mRNA**. *Nature* 2019, **571**:424-428.
12. Fu Y, Zhuang X: **m(6)A-binding YTHDF proteins promote stress granule formation**. *Nat Chem Biol* 2020, **16**:955-963.
13. Ries RJ, Pickering BF, Poh HX, Namkoong S, Jaffrey SR: **m(6)A governs length-dependent enrichment of mRNAs in stress granules**. *Nat Struct Mol Biol* 2023, **30**:1525-1535.
14. •• Shan T, Liu F, Wen M, Chen Z, Li S, Wang Y, Cheng H, Zhou Y: **m(6)A modification negatively regulates translation by switching mRNA from polysome to P-body via IGF2BP3**. *Mol Cell* 2023, **83**:4494-4508.

This work demonstrates that the m^{6}A methylation status of a transcript affects its subcellular localization and therefore translation regulation. Mechanistically, the m^{6}A reader IGF2BP3 was shown to recruit hypermethylated mRNAs into P-bodies, thereby making them inaccessible for the pool of polysomes that predominantly translate hypomethylated transcripts.

15. • Cheng Y, Xie W, Pickering BF, Chu KL, Savino AM, Yang X, Luo H, Nguyen DTT, Mo S, Barin E, *et al.*: **N6-Methyladenosine on mRNA facilitates a phase-separated nuclear body that suppresses myeloid leukemic differentiation**. *Cancer Cell* 2021, **39**:958-972 e958.

This article reported the formation of nuclear YTHDC1-m^{6}A condensates that were shown to promote AML by protecting leukemogenic transcripts from degradation. The findings support the concept of m^{6}A-dependent phase separation as a mechanism for transcript regulation.

16. Wang X, Liu C, Zhang S, Yan H, Zhang L, Jiang A, Liu Y, Feng Y, Li D, Guo Y, *et al.*: **N(6)-methyladenosine modification of MALAT1 promotes metastasis via reshaping nuclear speckles**. *Dev Cell* 2021, **56**:702-715 e708..
17. Wang S, Lv W, Li T, Zhang S, Wang H, Li X, Wang L, Ma D, Zang Y, Shen J, *et al.*: **Dynamic regulation and functions of mRNA m6A modification**. *Cancer Cell Int* 2022, **22**:48.
18. Helm M, Lyko F, Motorin Y: **Limited antibody specificity compromises epitranscriptomic analyses**. *Nat Commun* 2019, **10**:5669.
19. McIntyre ABR, Gokhale NS, Cerchietti L, Jaffrey SR, Horner SM, Mason CE: **Limits in the detection of m6A changes using MeRIP/m6A-seq**. *Sci Rep* 2020, **10**:6590.
20. Legrand C, Tuorto F, Hartmann M, Liebers R, Jacob D, Helm M, Lyko F: **Statistically robust methylation calling for whole-transcriptome bisulfite sequencing reveals distinct methylation patterns for mouse RNAs**. *Genome Res* 2017, **27**:1589-1596.
21. Thüring K, Schmid K, Keller P, Helm M: **Analysis of RNA modifications by liquid chromatography-tandem mass spectrometry**. *Methods* 2016, **107**:48-56.
22. • Koch J, Neuberger M, Schmidt-Dengler M, Xu J, Carneiro VC, Ellinger J, Kriegmair MC, Nuhn P, Erben P, Michel MS, *et al.*: **Reinvestigating the clinical relevance of the m(6)A writer METTL3 in urothelial carcinoma of the bladder**. *iScience* 2023, **26**:107300.

This study used a rigorous LC-MS/MS method to detect a pronounced global reduction of m^{6}A levels in bladder cancer. The results suggest that a global reduction of m^{6}A levels may be a general feature of tumor cells.

23. Garalde DR, Snell EA, Jachimowicz D, Sipos B, Lloyd JH, Bruce M, Pantic N, Admassu T, James P, Warland A, *et al.*: **Highly parallel direct RNA sequencing on an array of nanopores**. *Nat Methods* 2018, **15**:201-206.
24. Liu H, Begik O, Lucas MC, Ramirez JM, Mason CE, Wiener D, Schwartz S, Mattick JS, Smith MA, Novoa EM: **Accurate detection of m6A RNA modifications in native RNA sequences**. *Nat Commun* 2019, **10**:4079.
25. •• Liu C, Sun H, Yi Y, Shen W, Li K, Xiao Y, Li F, Li Y, Hou Y, Lu B, *et al.*: **Absolute quantification of single-base m6A methylation in the mammalian transcriptome using GLORI**. *Nat Biotechnol* 2023, **41**:355-366.

This study describes an unbiased, quantitative, transcriptome-wide, base-resolution m^{6}A sequencing technique that makes use of a chemical deamination approach for distinguishing m^{6}A from unmodified A. It has the potential to become the gold standard for the transcriptome-wide mapping of m^{6}A.

26. Yankova E, Blackaby W, Albertella M, Rak J, De Braekeleer E, Tsagkogeorga G, Pilka ES, Aspris D, Leggate D, Hendrick AG, *et al.*: **Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia**. *Nature* 2021, **593**:597-601.

27. Sun Y, Shen W, Hu S, Lyu Q, Wang Q, Wei T, Zhu W, Zhang J: **METTL3 promotes chemoresistance in small cell lung cancer by inducing mitophagy**. *J Exp Clin Cancer Res* 2023, **42**:65.

28. An X, Wu W, Yang L, Dong J, Liu B, Guo J, Chen J, Guo B, Cao W, Jiang Q: **ZBTB7C m6A modification incurred by METTL3 aberration promotes osteosarcoma progression**. *Transl Res* 2023, **259**:62-71.

29. Gao J, Fang Y, Chen J, Tang Z, Tian M, Jiang X, Tao C, Huang R, Zhu G, Qu W, *et al.*: **Methyltransferase like 3 inhibition limits intrahepatic cholangiocarcinoma metabolic reprogramming and potentiates the efficacy of chemotherapy**. *Oncogene* 2023, **42**:2507-2520.

30. Xu QC, Tien YC, Shi YH, Chen S, Zhu YQ, Huang XT, Huang CS, Zhao W, Yin XY: **METTL3 promotes intrahepatic cholangiocarcinoma progression by regulating IFIT2 expression in an m(6)A-YTHDF2-dependent manner**. *Oncogene* 2022, **41**:1622-1633.

31. Wang L, Yang Q, Zhou Q, Fang F, Lei K, Liu Z, Zheng G, Zhu L, Huo J, Li X, *et al.*: **METTL3-m(6)A-EGFR-axis drives lenvatinib resistance in hepatocellular carcinoma**. *Cancer Lett* 2023, **559**:216122.

32. Geula S, Moshitch-Moshkovitz S, Dominissini D, Mansour AA, Kol N, Salmon-Divon M, Hershkovitz V, Peer E, Mor N, Manor YS, *et al.*: **Stem cells. m6A mRNA methylation facilitates resolution of naïve pluripotency toward differentiation**. *Science* 2015, **347**:1002-1006.

33. • Poh HX, Mirza AH, Pickering BF, Jaffrey SR: **Alternative splicing of METTL3 explains apparently METTL3-independent m6A modifications in mRNA**. *PLoS Biol* 2022, **20**:e3001683.
This study identifies METTL3 as an essential gene in human cells, suggesting that m^6A is required for cellular viability. The results show that METTL3 knockout cell lines used alternative splicing to bypass CRISPR/Cas9-induced mutations and to express catalytically active isoforms.

34. Chang L, Ruiz P, Ito T, Sellers WR: **Targeting pan-essential genes in cancer: challenges and opportunities**. *Cancer Cell* 2021, **39**:466-479.

35. Rudd SG: **Targeting pan-essential pathways in cancer with cytotoxic chemotherapy: challenges and opportunities**. *Cancer Chemother Pharm* 2023, **92**:241-251.

36. Klein IA, Boija A, Afeyan LK, Hawken SW, Fan M, Dall'Agnese A, Oksuz O, Henninger JE, Shrinivas K, Sabari BR, *et al.*: **Partitioning of cancer therapeutics in nuclear condensates**. *Science* 2020, **368**:1386-1392.

37. Patel A, Mitrea D, Namasivayam V, Murcko MA, Wagner M, Klein IA: **Principles and functions of condensate modifying drugs**. *Front Mol Biosci* 2022, **9**:1007744.

38. Mitrea DM, Mittasch M, Gomes BF, Klein IA, Murcko MA: **Modulating biomolecular condensates: a novel approach to drug discovery**. *Nat Rev Drug Discov* 2022, **21**:841-862.

39. •• Guirguis AA, Ofir-Rosenfeld Y, Knezevic K, Blackaby W, Hardick D, Chan YC, Motazedian A, Gillespie A, Vassiliadis D, Lam EYN, *et al.*: **Inhibition of METTL3 results in a cell-intrinsic interferon response that enhances antitumor immunity**. *Cancer Discov* 2023, **13**:2228-2247.
This report presents an improved METTL3 inhibitor and shows that it induces a cell-intrinsic interferon response, which enhanced antitumor immunity. This suggests a novel immunomodulatory mechanism for m^6A.

40. Zhang L, Li Y, Zhou L, Zhou H, Ye L, Ou T, Hong H, Zheng S, Zhou Z, Wu K, *et al.*: **The m6A reader YTHDF2 promotes bladder cancer progression by suppressing RIG-I-mediated immune response**. *Cancer Res* 2023, **83**:1834-1850.

41. Pan Y, Chen H, Zhang X, Liu W, Ding Y, Huang D, Zhai J, Wei W, Wen J, Chen D, *et al.*: **METTL3 drives NAFLD-related hepatocellular carcinoma and is a therapeutic target for boosting immunotherapy**. *Cell Rep Med* 2023, **4**:101144.

42. Chen H, Pan Y, Zhou Q, Liang C, Wong CC, Zhou Y, Huang D, Liu W, Zhai J, Gou H, *et al.*: **METTL3 inhibits antitumor immunity by targeting m(6)A-BHLHE41-CXCL1/CXCR2 axis to promote colorectal cancer**. *Gastroenterology* 2022, **163**:891-907.

43. Yu H, Liu J, Bu X, Ma Z, Yao Y, Li J, Zhang T, Song W, Xiao X, Sun Y, *et al.*: **Targeting METTL3 reprograms the tumor microenvironment to improve cancer immunotherapy**. *Cell Chem Biol* 2023, **31**:776-791.

44. Vu LP, Pickering BF, Cheng Y, Zaccara S, Nguyen D, Minuesa G, Chou T, Chow A, Saletore Y, MacKay M, *et al.*: **The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells**. *Nat Med* 2017, **23**:1369-1376.

45. Li Z, Peng Y, Li J, Chen Z, Chen F, Tu J, Lin S, Wang H: **N(6)-methyladenosine regulates glycolysis of cancer cells through PDK4**. *Nat Commun* 2020, **11**:2578.

46. Lin S, Liu J, Jiang W, Wang P, Sun C, Wang X, Chen Y, Wang H: **METTL3 promotes the proliferation and mobility of gastric cancer cells**. *Open Med (Wars)* 2019, **14**:25-31.

47. Zhao X, Cui L: **Development and validation of a m(6)A RNA methylation regulators-based signature for predicting the prognosis of head and neck squamous cell carcinoma**. *Am J Cancer Res* 2019, **9**:2156-2169.

48. Chen M, Wei L, Law CT, Tsang FH, Shen J, Cheng CL, Tsang LH, Ho DW, Chiu DK, Lee JM, *et al.*: **RNA N6-methyladenosine methyltransferase-like 3 promotes liver cancer progression through YTHDF2-dependent posttranscriptional silencing of SOCS2**. *Hepatology* 2018, **67**:2254-2270.

49. Lin S, Choe J, Du P, Triboulet R, Gregory RI: **The m(6)A methyltransferase METTL3 promotes translation in human cancer cells**. *Mol Cell* 2016, **62**:335-345.

50. Dahal U, Le K, Gupta M: **RNA m6A methyltransferase METTL3 regulates invasiveness of melanoma cells by matrix metallopeptidase 2**. *Melanoma Res* 2019, **29**:382-389.

51. Taketo K, Konno M, Asai A, Koseki J, Toratani M, Satoh T, Doki Y, Mori M, Ishii H, Ogawa K: **The epitranscriptome m6A writer METTL3 promotes chemo- and radioresistance in pancreatic cancer cells**. *Int J Oncol* 2018, **52**:621-629.

52. Cai J, Yang F, Zhan H, Situ J, Li W, Mao Y, Luo Y: **RNA m(6)A methyltransferase METTL3 promotes the growth of prostate cancer by regulating hedgehog pathway**. *Onco Targets Ther* 2019, **12**:9143-9152.

53. Hua W, Zhao Y, Jin X, Yu D, He J, Xie D, Duan P: **METTL3 promotes ovarian carcinoma growth and invasion through the regulation of AXL translation and epithelial to mesenchymal transition**. *Gynecol Oncol* 2018, **151**:356-365.

54. Cheng M, Sheng L, Gao Q, Xiong Q, Zhang H, Wu M, Liang Y, Zhu F, Zhang Y, Zhang X, *et al.*: **The m(6)A methyltransferase METTL3 promotes bladder cancer progression via AFF4/NF-κB/MYC signaling network**. *Oncogene* 2019, **38**:3667-3680.

55. Zhao S, Liu J, Nanga P, Liu Y, Cicek AE, Knoblauch N, He C, Stephens M, He X: **Detailed modeling of positive selection improves detection of cancer driver genes**. *Nat Commun* 2019, **10**:3399.

56. Cai X, Wang X, Cao C, Gao Y, Zhang S, Yang Z, Liu Y, Zhang X, Zhang W, Ye L: **HBXIP-elevated methyltransferase METTL3 promotes the progression of breast cancer via inhibiting tumor suppressor let-7g**. *Cancer Lett* 2018, **415**:11-19.

57. Shi Y, Zheng C, Jin Y, Bao B, Wang D, Hou K, Feng J, Tang S, Qu X, Liu Y, *et al.*: **Reduced expression of METTL3 Promotes metastasis of triple-negative breast cancer by m6A methylation-mediated COL3A1 up-regulation**. *Front Oncol* 2020, **10**:1126.

58. Uddin MB, Roy KR, Hosain SB, Khiste SK, Hill RA, Jois SD, Zhao Y, Tackett AJ, Liu YY: **An N(6)-methyladenosine at the transited codon 273 of p53 pre-mRNA promotes the expression of R273H mutant protein and drug resistance of cancer cells**. *Biochem Pharm* 2019, **160**:134-145.

59. Deng R, Cheng Y, Ye S, Zhang J, Huang R, Li P, Liu H, Deng Q, Wu X, Lan P, *et al.*: **m(6)A methyltransferase METTL3 suppresses**

colorectal cancer proliferation and migration through p38/ERK pathways. *Onco Targets Ther* 2019, **12**:4391-4402.

60. Wang K, Jiang L, Zhang Y, Chen C: **Progression of thyroid carcinoma is promoted by the m6A methyltransferase METTL3 through regulating m(6)A methylation on TCF1**. *Onco Targets Ther* 2020, **13**:1605-1612.

61. He J, Zhou M, Yin J, Wan J, Chu J, Jia J, Sheng J, Wang C, Yin H, He F: **METTL3 restrains papillary thyroid cancer progression via m(6)A/c-Rel/IL-8-mediated neutrophil infiltration**. *Mol Ther* 2021, **29**:1821-1837.

62. Liu J, Eckert MA, Harada BT, Liu SM, Lu Z, Yu K, Tienda SM, Chryplewicz A, Zhu AC, Yang Y, *et al*.: **m(6)A mRNA methylation regulates AKT activity to promote the proliferation and tumorigenicity of endometrial cancer**. *Nat Cell Biol* 2018, **20**:1074-1083.

63. Li X, Tang J, Huang W, Wang F, Li P, Qin C, Qin Z, Zou Q, Wei J, Hua L, *et al*.: **The M6A methyltransferase METTL3: acting as a tumor suppressor in renal cell carcinoma**. *Oncotarget* 2017, **8**:96103-96116.

64. Yue B, Song C, Yang L, Cui R, Cheng X, Zhang Z, Zhao G: **METTL3-mediated N6-methyladenosine modification is critical for epithelial-mesenchymal transition and metastasis of gastric cancer**. *Mol Cancer* 2019, **18**:142.