Novel insight into the regulatory roles of diverse RNA modifications: Re-defining the bridge between transcription and translation

Hanhan Shi[1,2†], Peiwei Chai[1,2†], Renbing Jia[1,2*] and Xianqun Fan[1,2*]

Abstract

RNA modifications can be added or removed by a variety of enzymes that catalyse the necessary reactions, and these modifications play roles in essential molecular mechanisms. The prevalent modifications on mRNA include N6-methyladenosine (m^6A), N1-methyladenosine (m^1A), 5-methylcytosine (m^5C), 5-hydroxymethylcytosine (hm^5C), pseudouridine (Ψ), inosine (I), uridine (U) and ribosemethylation (2'-O-Me). Most of these modifications contribute to pre-mRNA splicing, nuclear export, transcript stability and translation initiation in eukaryotic cells. By participating in various physiological processes, RNA modifications also have regulatory roles in the pathogenesis of tumour and non-tumour diseases. We discussed the physiological roles of RNA modifications and associated these roles with disease pathogenesis. Functioning as the bridge between transcription and translation, RNA modifications are vital for the progression of numerous diseases and can even regulate the fate of cancer cells.

Keywords: RNA modifications, m^6A, m^1A, m^5C, diseases

Introduction

In the 1950s, the first RNA nucleoside modification was identified [1]; since then, researchers have focused on updating the understanding of RNA modifications. At the very beginning, the 5'cap and the poly(A) tail, which represent cap and tail modifications, respectively, were discovered. However, with the limitations of technology, modifications of eukaryotic mRNA ends were considered the only post-transcriptional alterations to mRNA for a while. Fortunately, this situation did not last for a long time. Internal mRNA modifications have been investigated in succession in the last 50 years. The revealed mRNA modifications included but were not limited to N6-methyladenosine (m^6A), N1-methyladenosine (m^1A), 5-methylcytosine (m^5C), 5-hydroxymethylcytosine (hm^5C), pseudouridine (Ψ), inosine (I), uridine (U) and ribose-methylation (2'-O-Me) (Figs. 1 and 2). m^6A is the most abundant modification and was therefore thoroughly investigated [5].

Analogous to mRNA modification, we also identified many modifications on transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), such as queuosine (Q) [6]. Eukaryotic tRNAs contain, on average, over 10 modifications per molecule. From elementary isomerization or methylation to complicated modifications of ring structures, the number of tRNA modifications is the largest and has the widest chemical variety. Moreover, there are over 200 modifications on human rRNAs. Thus, their less complicated nature and greater abundance led to more investigations of tRNAs and rRNAs, even beyond mRNAs. Early studies have demonstrated that this variety of modifications leads to extra cellular functions for diverse RNA species [7].

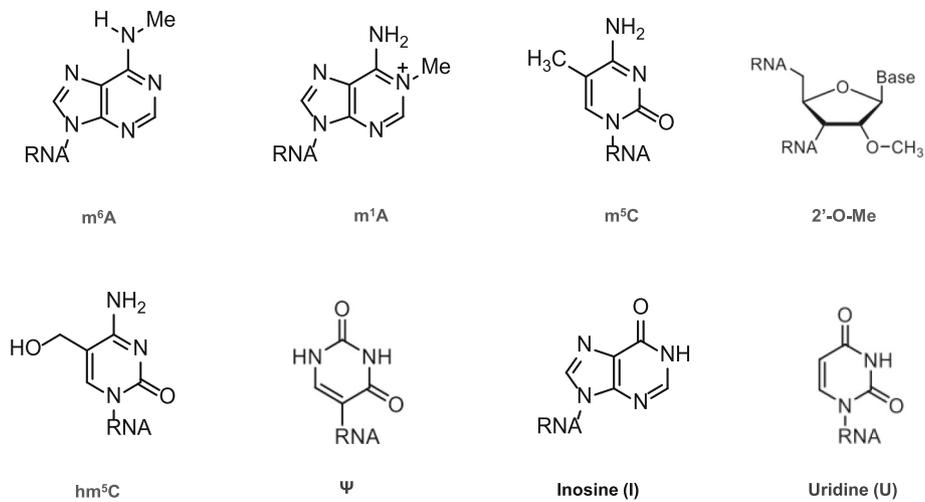* Correspondence: renbingjia@sjtu.edu.cn; fanxq@sjtu.edu.cn
†Hanhan Shi and Peiwei Chai contributed equally to this work.
1Department of Ophthalmology, Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, Shanghai 20025, P.R. China
Full list of author information is available at the end of the article

Fig. 1 Chemical structures of mRNA modifications. Chemical structures in eukaryotic mRNA including m⁶A, m¹A, m⁵C, hm⁵C, Ψ, I, U and 2'-O-Me

The regulatory role of RNA modifications

Modifications on different RNAs were found to regulate various cellular processes. Researchers demonstrated that these modifications can initiate translation, stabilize transcripts, splice pre-mRNA, facilitate nuclear export, etc. [8–12]. With respect to RNA modifications and technological advances in high-throughput sequencing and mass spectrometry, the mechanisms of different cellular processes influenced by RNA modifications are underexplored, including the less ubiquitous modifications on rare
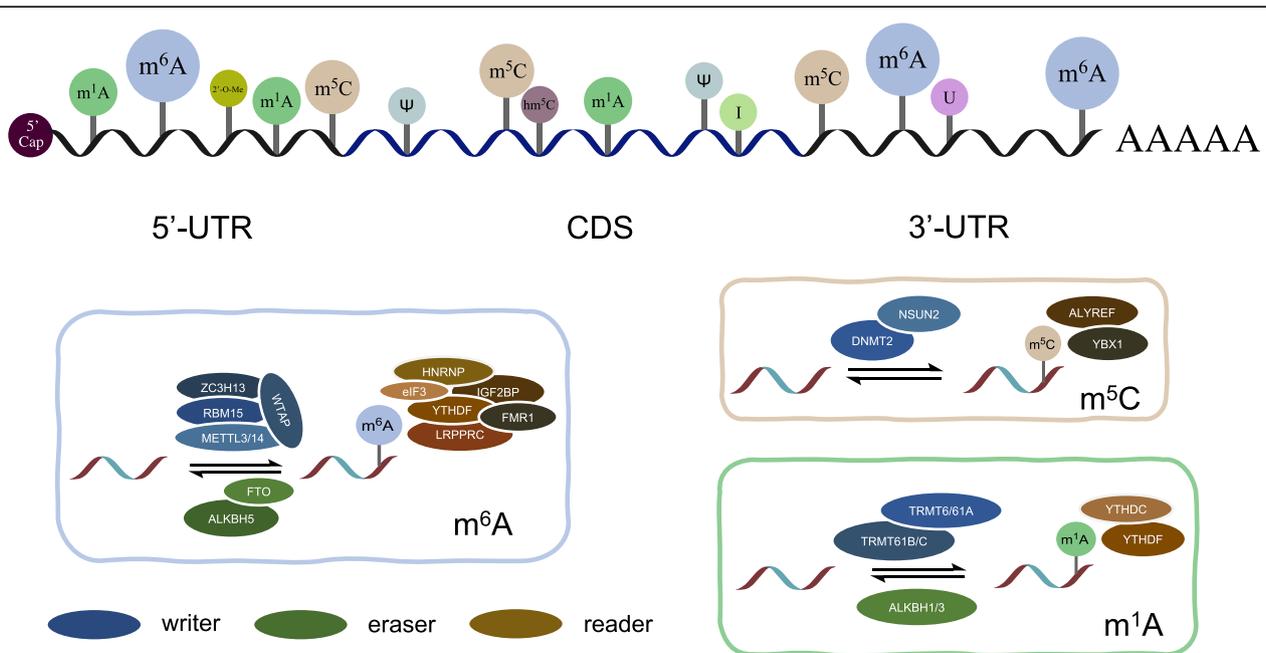
Fig. 2 Locations of chemical modifications in mRNA. Chemical RNA modifications are shown in mRNA with their approximate distribution in transcripts. m⁶A with a widespread distribution prefers to be located in the consensus motif in the 3'UTRs as well as the 5'UTRs, which closely correlate with translation. Although m¹A-containing mRNA is 10 times less common than m⁶A-containing mRNA, m¹A is discovered in every segment of mRNA, including the 5'UTRs, CDS and 3'UTRs and mostly in highly structured 5'UTRs. Analogous to m¹A, m⁵C can occur in coding and non-coding regions of mRNA, especially in GC-rich regions. Nevertheless, m⁵C within different positions regulates transcription differently. Tet-family enzymes prefer to oxidize m⁵C modifications in coding regions, so hm⁵C has a greater possibility of being present in CDS. Subsequently, Ψ is demonstrated to have a diversified location, whereas I is present at a large number of sites in the CDS, and U accumulates in 3'UTRs. 2'-O-Me focuses on decorating specific regions of mRNA that encode given amino acids. Additionally, as reversible modifications, most have their own readers, writers and erasers

RNA species. tRNAs, which have the greatest number of types of different chemical modifications, regulate molecular mechanisms by selecting and protecting the reading framework, promoting tRNA decoding capability as well as changing codon-anti-codon connections [13–17]. Moreover, the functions of 2'-O-Me, Ψ and m^5C, which are abundant in rRNA, have been investigated in detail. Without any doubt, mRNA modifications play roles in modulating molecular mechanisms. Subsequently, RNA modifications contribute to tumorigenesis by regulating cell survival, differentiation, migration and drug resistance [18].

m^6A RNA modification
Introduction to m^6A RNA modification
m^6A accounts for approximately 0.2~0.6% of total adenosines in mammalian RNA [2, 5]. General m^6A modifications occur in mammals, plants, bacteria and even other types of eukaryotic RNA [19–22]. In addition to their widespread distribution, there is no less than 1-2 methylated adenosines in every single mRNA [23]. Studies have reported that m^6A is located in the 3' untranslated region (3'UTR), predominantly in a consensus motif, GGm^6ACU [24–26]. Recently, m^6A was also found in the 5' untranslated region (5'UTR), a region that closely correlates with translation. It has been reported that methylated adenosine in the 5'UTR of mRNA can support cap-independent translation commencement and can increase translation [27, 28].

As a reversible mRNA modification, m^6A has its own writers, readers and erasers. Methyltransferase-like 3 (METTL3) was the first demonstrated m^6A writer [29]. In addition to METTL3, other proteins possessing methyltransferase (MTase) capability were recently identified, including methyltransferase-like 14 (METTL14), Wilms tumour 1-associated protein (WTAP), RNA-binding motif protein 15 (RBM15), KIAA 1429 and zinc finger CCCH-type containing 13 (ZC3H13) [30–33]. By binding to mRNA, readers, such as members of the YT521-B homology (YTH) domain family of proteins (YTHDF1, YTHDF2, YTHDF3, YTHDC1 and YTHDC2) and heterogeneous nuclear ribonucleoprotein (HNRNP) proteins (HNRNPA2B1 and HNRNPC) can execute the physiological functions of the modification [8, 10, 12, 34–38]. Additionally, eukaryotic initiation factor 3 (eIF3), insulin-like growth factor 2 mRNA-binding proteins (IGF2BP1, IGF2BP2 and IGF2BP3), fragile X mental retardation 1 (FMR1) and leucine-rich pentatricopeptide repeat-containing (LRPPRC) all can read m^6A modifications [39, 40]. Both fat mass and obesity-associated protein (FTO) and alkB homologue 5 (ALKBH5) are erasers of m^6A modifications [11, 41, 42].

Regulatory role of m^6A RNA modification in molecular functions
Accumulation of pre-mRNA and diminution of mature mRNA in cyclo-leucine-treated avian sarcoma virus-infected cells and neplanocin A (NPC)-treated SV40 RNA demonstrate that m^6A is essential in pre-mRNA splicing [43, 44]. Both cyclo-leucine and NPC are inhibitors of methylation that can be used to investigate m^6A [45, 46]. Subsequently, MTases and demethylases might be involved in regulating RNA splicing. By changing RNA structure and regulating the combination of RNA and reader proteins, HNRNPC can modulate the splicing of m^6A-containing mRNAs [10]. More recently, by relying on the RGG region in the low-complication region of HNRNPG, a reader was reported to cooperate with modified pre-mRNA and the phosphorylated C-terminal domain of RNA polymerase II to modulate splicing [47]. Moreover, FTO is vital to mRNA splicing because it prefers to bind to introns of nascent mRNA [48]. Another splicing-related eraser is ALKBH5. Immunofluorescence analysis revealed that ALKBH5 was tightly related to splicing factors [11].

Writers, readers and erasers can all regulate mRNA export. By modulating the clock genes Per2 and Arntl, METTL3 regulates the export of mature mRNA [49]. By interacting with SRSF3 and regulating the combination of SRSF3 and NXF1 on RNA, YTHDC1 mediates the export of modified mRNA [50]. Subsequently, knockdown of ALKBH5 leads to acceleration of mRNA export, suggesting that m^6A is essential to regulating mRNA export [11].

AU-rich element (ARE), iron-responsive element (IRE) and cytoplasmic polyadenylation element (CPE) represent functional domains and are responsible for mRNA decay in 3'UTRs [51]. Coincidentally, m^6A accumulates in 3'UTRs. Thus, the neighbouring sites of m^6A and Hu antigen R (HuR), which is supposed to bind ARE to increase the stability of mRNA, lead to weak HuR function and mRNA instability [52]. However, ELAV1/HuR, a potential m^6A-binding protein, can stabilize transcripts with the cooperation of the ARE domain [53]. Subsequently, it was reported that the stability of mRNA was decreased slightly in cells lacking ALKBH5 [11].

The YTH domain family of proteins has a conserved m^6A-binding pocket so that these proteins can tightly bind to m^6A in a consensus sequence and directly transcribe the molecule [12, 26, 34–38]. Specifically, YTHDF2 accelerates mRNA decay by transferring RNA from the translatable pool to processing bodies [12]. Under heat shock conditions, dysfunction of FTO in 5'UTRs, which is regulated by YTHDF2, contributes to the promotion of cap-independent translation [28]. Moreover, YTHDF1 can increase the efficiency of translation by binding m^6A [37]. Subsequently, YTHDF3 can regulate translation by both interacting with ribosomal proteins with bound YTHDF1 and by decaying the translation-related mRNA region with bound YTHDF2 [54, 55]. However, METTL3 can regulate translation flexibly because it can either recruit eIF3 to the initiation

complex directly to increase translation or can inhibit translation efficiency [56, 57]. The translation efficiency is increased when METTL3 is knocked out in mouse embryonic stem cells (mESCs) and embryoid bodies (EBs) [57] (Fig. 3).

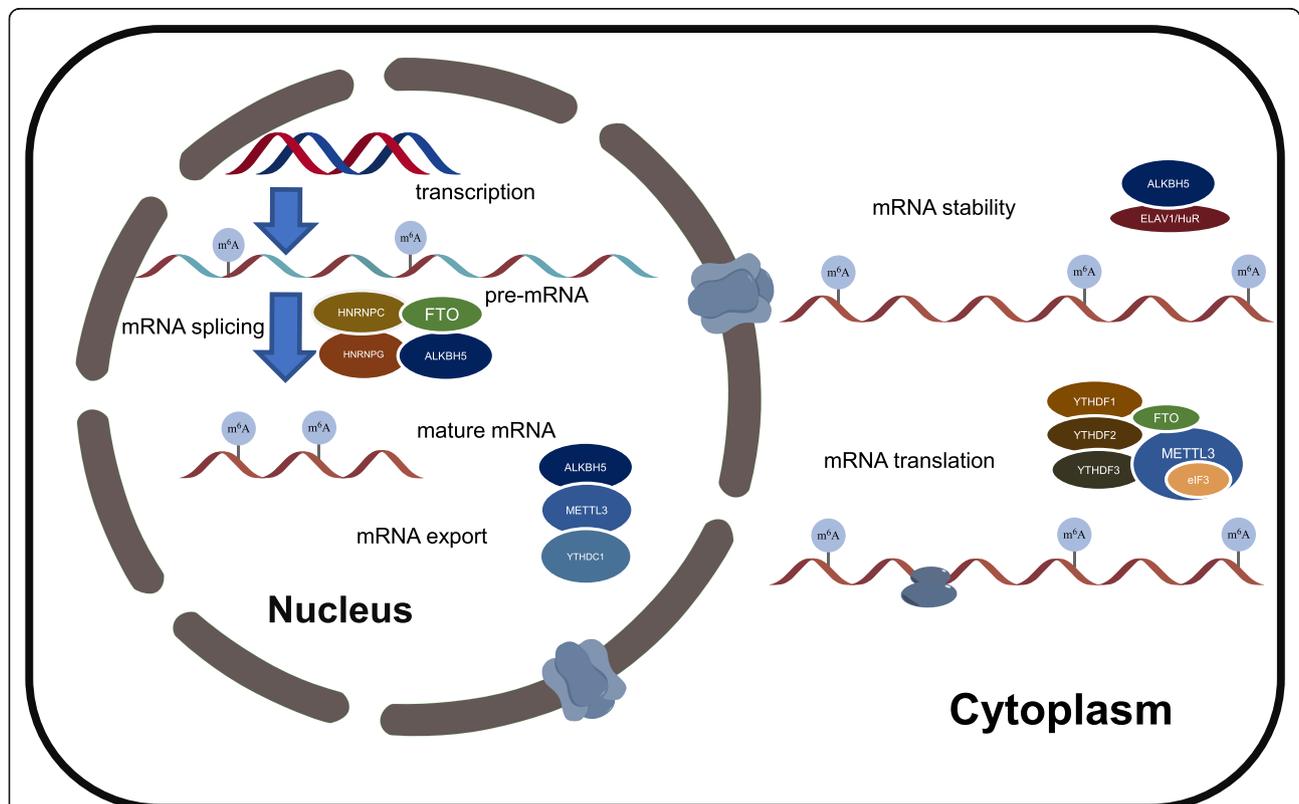## m^1A RNA modification
Introduction of m^1A RNA modification

m^6A has been reported to occur in DNA from a minor cluster of microorganisms and in RNA from an extensive range of organisms, and additionally, m^1A was identified in the 1960s [58]. Rather than accumulating in mRNA, m^1A is predominant in tRNA and rRNA, but we recently determined that it also exists in mRNA [59, 60]. However, m^1A-containing mRNA is 10 times less common than m^6A-containing mRNA [61, 62]. In tRNA and rRNA, m^1A conserves the tertiary structure and affects translation [63, 64]. In mRNA, m^1A has been discovered in every mRNA segment, including the coding sequence (CDS), 5'UTR and 3'UTR, although it is mostly found in the highly structured 5'UTR [62]. As a result, the

location of the m^1A methylated atom determines the function and mechanism of this kind of modification.

Because the distribution of m^1A is imbalanced, the large number of m^1A modifications on tRNA results in more tRNA m^1A MTases than writers on mRNA. However, TRMT6/61A recognized a T-loop-like structure with a GUUCRA tRNA-like motif in mRNAs and decorated it with the m^1A modification, TRMT61B installed m^1A in mt-mRNA transcripts, and TRMT10C methylated the 1374 position of ND5 mt-mRNA [65, 66]. All of these phenomena contribute to tRNA m^1A MTases and can function as mRNA writers. By binding to m^1A-bearing RNA, YTHDF1, YTHDF2, YTHDF3 and YTHDC1 act as readers [67]. Subsequently, similar to ALKBH5 functioning as an eraser for m^6A, ALKBH1 and ALKBH3 were able to demethylate m^1A mRNA modifications [62, 68].

Regulatory role of the m^1A RNA modification in molecular functions

It has been reported that m^1A methylation occurs in highly structured or GC-rich regions of 5'UTRs (which

Fig. 3 m^6A RNA modification regulates physiological processes in cell. m^6A RNA modification in mRNA plays an essential role in cellular processes, including mRNA splicing, mRNA export, mRNA stability and mRNA translation. Both readers (HNRNPC and HNRNPG) and erasers (FTO and ALKBH5) can modulate the splicing of mRNA. After splicing and combination, pre-mRNA evolves into mature mRNA. Regulated by ALKBH5, METTL3 and YTHDC1, mature mRNA is exported from the nucleus to the cytoplasm. Once exported to the cytoplasm, both ALKBH5 and ELAV1/HuR can maintain mRNA stability. Finally, numerous enzymes contribute to the process of translation. YTHDF1, YTHDF2, YTHDF3, FTO and METTL3 together with eIF3 can regulate translation with different mechanisms individually

Shi *et al. Molecular Cancer*   (2020) 19:78

Page 5 of 17

is also the most frequent location) and may modify the predicted secondary structure, which hints at the potential of m^1A to alter mRNA structural stability [61, 62].Moreover, m^1A methylation can not only increase translation by decreasing the binding of the releasing factor but also prevent effective translation of m^1A-containing CDS in mt-mRNA [26, 65]. Ultimately, it has been reported that the protein level is higher when a transcript carries the m^1A modification around the initiation codon [69].

m^5C RNA modification
Introduction of the m^5C RNA modification
m^5C is a long-standing DNA modification that is essential for gene expression and epigenetic regulation [70, 71]. However, it can also be found in RNA. Although the m^5C RNA modification can appear in both coding and non-coding regions, it has been reported to accumulate in the UTRs of mRNA and especially prefers to be located in GC-rich regions [72]. Since a number of studies have investigated the function of m^5C in specific mRNAs, we concluded that m^5C modifications in different locations (5'UTRs, 3'UTRs, coding regions) exert different transcriptional regulation activities [73].

It was revealed that m^5C RNA modifications are catalysed by the NOL1/NOP2/SUN domain (NSUN) family of proteins (NSUN1, NSUN2, NSUN3, NSUN4, NSUN5, NSUN6 and NSUN7) as well as the DNA methyltransferase (DNMT) homologue DNMT2 [74–76]. However, among such diversified writers, only NSUN2 can install m^5C on mRNA because rest of these proteins are writers of tRNAs and rRNAs. Subsequently, Aly/REF export factor (ALYREF), a specific mRNA m^5C-binding protein that can read modifications, was identified as a reader of m^5C [77]. According to liquid chromatography-tandem mass spectrometry analysis, YBX1 was defined as the other m^5C reader that can maintain the stability of target mRNA [78]. Knowledge is limited about the protein factors responsible for removing modifications (Table 1).

Regulatory role of the m^5C RNA modification in molecular functions
ALYREF, the reader of m^5C, can adjust the export of transcripts by recognizing a unique RNA-binding motif [77]. Subsequently, NSUN2 adds m^5C to both p27

mRNA at cytosine C64 in the 5'UTR and p21 mRNA in the 3'UTR [79, 80]. Deleting NSUN2 in human diploid fibroblasts (HDFs) can induce the elevation of p27, and overexpressing NSUN2 results in contrasting outcomes [79]. These results suggest that the m^5C catalysed by NSUN2 in the 5'UTRs can limit the translation of p27. However, the m^5C modifications added by NSUN2 to the 3'UTRs of p21 mRNA coordinate with the m^6A modifications added by METTL3/METTL14 together to enhance the expression of p21 [80]. With regard to m^5C modification in mRNA coding regions, it was revealed that in both bacterial whole-cell extracts and HeLa cell extracts, m^5C could diminish translation significantly [27, 81]. Moreover, we demonstrated that when the m^5C modification was present on interleukin-17A (IL-17A) mRNA, this modification could promote the translation of IL-17A [82]. The results of the above investigations revealed that the m^5C RNA modification affects the expression of proteins by regulating both translation efficiency and transcript export (Table 2).

Other RNA modifications
hm^5C
m^5C can be oxidized into hm^5C via the function of the Tet-family enzymes [91–93]. Moreover, hMeRIP-seq showed that Tet-family enzymes prefer to oxidize m^5C modifications in coding regions; these results indicate that hm^5C is highly likely to be located in the introns and exons of coding transcripts. However, in contrast to m^5C methylation in the coding regions of mRNA, which plays a negative role in translation, hm^5C tends to associate with translation activation in Drosophila [69].

Ψ
As hm^5C is analogous to the oxidization of m^5C, Ψ is produced by the isomerization of U. Ψ is the most abundant RNA modification and prefers to accumulate in tRNA and rRNA; however, it has also been reported to be present on mRNA and snRNA [94, 95]. Interestingly, the number of Ψ sites in mRNA ranges from 96 to 2084 in humans [84, 96–98].

However, by regulating U2 auxiliary factor (U2AF), Ψ, which is near the 3' splice site in the polypyrimidine tract, prevents pre-mRNA splicing [83]. Expression of heat shock-induced Pus7-dependent pseudouridylated

Table 1 Writers, readers and erasers of the predominant mRNA modifications

RNA modification	Writers	Readers	Erasers
m^6A	METTL3; METTL14; WTAP; RBM15; ZC3H13	YTH domain family of proteins (YTHDF1, YTHDF2, YTHDF3, YTHDC1 and YTHDC2); HNRNP (HNRNPA2B1 and HNRNPC); eIF3; IGF2BP (IGF2BP1, IGF2BP2, and IGF2BP3); FMR1; LRPPRC	FTO; ALKBH5
m^1A	TRMT6/61A; TRMT61B; TRMT10C	YTHDF1; YTHDF2; YTHDF3; YTHDC1	ALKBH1; ALKBH3
m^5C	NSUN2; DNMT2	ALYREF; YBX1	N.A.

Shi *et al. Molecular Cancer*   (2020) 19:78

Page 6 of 17

Table 2 mRNA modifications regulate the physiological process from transcription to translation

Modifications	Process	Enzymes involved	Description	Ref
m^{6}A RNA modification	mRNA splicing	HNRNPC	HNRNPC modulates the splicing of mRNAs by changing RNA structure and regulating the combination of RNA and reader	[10]
		HNRNPG	HNRNPG cooperates with modified pre-mRNA and the phosphorylated C-terminal domain of RNA polymerase II to regulate splicing	[47]
		FTO	FTO prefers to bind to introns of nascent mRNA	[48]
		ALKBH5	ALKBH5 relates to splicing factors tightly according to the analysis of immunofluorescence	[11]
	mRNA export	METTL3	METTL3 regulates the export of mature mRNA by modulating clock genes Per2 and Arntl	[49]
		YTHDC1	YTHDC1 mediates the export of decorated mRNA by interacting with SRSF3 and regulating the combination of SRSF3 an NXF1 on RNA	[50]
		ALKBH5	Knockdown of ALKBH5 leads to acceleration in mRNA export	[11]
	mRNA stability	ALKBH5	The stability of mRNA was decreased slightly in RNA lacking ALKBH5	[11]
		N.A.	Neighbouring sites of m^{6}A and HuR weaken the function of HuR and increase the instability of mRNA	[52]
		N.A.	ELAV1/HuR, which is one of m^{6}A-binding proteins and stabilizes transcripts with the cooperation of the ARE domain	[53]
	mRNA translation	YTHDF2	YTHDF2 regulates translation by transferring the bound RNA from the translatable pool to processing bodies to promote mRNA decay	[12]
			YTHDF2 induces the dysfunction of FTO in the 5'UTRs and contribute to promoting cap-independent translation	[28]
		YTHDF1	YTHDF1 increases the efficiency of translation by binding to m^{6}A	[37]
		YTHDF3	YTHDF3 interacts with ribosomal proteins along with YTHDF1 to regulate translation	[54]
			YTHDF3 decays of convinced translation related region in mRNA together with YTHDF2	[55]
		METTL3	When knocking out METTL3 in mESCs and Ebs, the translation efficiency is increased	[57]
			METTL3 recruits eIF3 to the initiation complex directly and enhance translation level	[56]
m^{1}A RNA modification	mRNA stability	N.A.	m^{1}A in highly structured or GC-rich regions of 5'UTRs alters mRNA structural stability by modifying the predicted secondary structure	[61, 62]
	mRNA translation	N.A.	m^{1}A upregulated translation by depressing binding of releasing factor	[26]
		N.A.	m^{1}A prevents effective translation of CDS in mt-mRNA	[65]
		N.A.	The protein level would be superior when the transcript was modified by m^{1}A at/around the initiation codon	[69]
m^{5}C RNA modification	mRNA export	ALYREF	ALYREF adjusts the export of transcripts by recognizing the unique RNA-binding motif	[77]
	mRNA translation	NSUN2	Deleting NSUN2 in HDFs can induce the elevation of p27, and overexpressing NSUN2 induces the opposite outcome	[79]
			m^{5}C catalysed by NSUN2 in 3'UTRs of p21 mRNA coordinates with m^{6}A methylated by METTL3/METTL14 together to enhance p21 expression	[80]
		N.A.	Translation diminishes significantly in both bacterial whole-cell extracts and HeLa cell extracts when m^{5}C modifies the coding regions of mRNA	[27, 81]
		N.A.	m^{5}C found on IL-17A mRNA can promote the translation of IL-17A	[82]
Other hm^{5}C	mRNA translation	N.A.	hm^{5}C associates with translation activation in Drosophila	[69]
Ψ	mRNA splicing	N.A.	Ψ, which is near the 3' splice site in the polypyrimidine tract, prevents pre-mRNA splicing by regulating U2AF	[83]
	mRNA stability	N.A.	The higher expression of heat shock-induced Pus7-dependent pseudouridylated transcripts in wild-type yeast than in Pus7-knockdown yeast indicates that Ψ has the capability to maintain stability of RNA	[84]
	mRNA translation	N.A.	Compared to U modifications located at similar sequences, Ψ-containing mRNA indicates an increase in translation levels of approximately 25%	[84]
		N.A.	Ψ doubles the expression of an unmodified transcript	[85]
		N.A.	When a separate Ψ modifies the special position of codon "UUU", mRNA translation can be limited	[81]

Shi *et al. Molecular Cancer*   (2020) 19:78

Page 7 of 17

Table 2 mRNA modifications regulate the physiological process from transcription to translation *(Continued)*

Modifications	Process	Enzymes involved	Description	Ref
I	mRNA structure	N.A.	I fastens pairs of nucleotides to influence the native secondary structure of mRNA	[86]
	mRNA translation	N.A.	Guanosine, adenosine and uracil are the products decoded from I by the translation machinery	[87]
U	Protein expression	N.A.	Protein level alterations accompany C-to-U editing of RNA	[88]
2'-O-Me	Viral RNA infection	N.A.	2'-O-Me-modified viral RNA disrupts native host antiviral responses by escaping suppression mediated by IFIT	[89]
	mRNA translation	N.A.	2'-O-Me modifies specific regions of mRNA that are translated to glutamate, lysine and glutamine, hinting that 2'-O-Me has the potential to affect translation efficiency	[90]

transcripts is higher in wild-type yeast than in Pus7-knockdown yeast and indicates that Ψ has the capability to maintain RNA stability [84]. Nevertheless, modifications were examined at similar sequences, and compared to U-containing mRNA, Ψ-containing mRNA experienced an increase in translation by approximately 25% [84]. Such modifications could double the expression of translation when compared to blank control transcript without any modification [85]. Although Ψ can promote translation and enhance the lifespan of RNA, it has negative effects on protein expression [85]. It has been reported that Ψ-containing mRNA exhibits a 30% decrease in protein expression. Specifically, bacterial mRNA translation can be limited when a separate Ψ modification is present at a given position of codon "UUU", especially at the third codon position [81]. Moreover, both in vitro and in vivo, the Ψ modification might change the nonsense codons into sense codons [99, 100]. Above all, some of these investigations were conducted by Ψ in artificial mRNA, and the function of Ψ in biological mRNA has yet to be elucidated.

I and U
Catalysed by adenosine or cytidine deaminating enzymes, RNA editing is a kind of programmed alteration [101]. However, rather than permanent DNA mutations or reversible RNA modifications, RNA editing has its own limited lifespan and results in more permanent modification [102].

Adenosine-to-inosine RNA editing (A-to-I editing), also called I, is catalysed by adenosine deaminases acting on RNA (ADARs) [101, 103, 104]. Recently, 1741 I sites have been reported in CD regions of transcripts from RNA-seq data of different human tissues [105]. Moreover, it has been reported that ADAR1 and ADAR2 act only on double-stranded regions, which limits the areas of mRNA that I can modify [106]. I can fasten pairs of nucleotides; thus, this widespread modification in metazoan mRNA can influence the native secondary structure of mRNA [86]. An in vitro translation system was

implemented to scientifically test the decoding of I, revealing that guanosine, adenosine and uracil are the products decoded from I by translation machinery [87].

However, with regard to cytidine-to-uridine RNA editing (C-to-U editing), also called U, it has been reported that U accumulates in 3'UTRs, and over 70 new sites have been discovered by transcriptome-wide research [88, 107]. Subsequently, after exploring several intestinal mRNAs, it was revealed that the protein level is altered by C-to-U editing of RNA [88]. However, there is little research on the relationship between the expression of transcripts and U. The biological influence of U has yet to be investigated.

2'-O-Me
Unlike how I and U are modifications on a base, 2'-O-Me is methylation of ribose at the 2' position [59]. It was revealed that by escaping the suppression mediated by IFN-induced proteins with tetratricopeptide repeats (IFIT), 2'-O-Me-modifiedviral RNA disrupts native host antiviral responses [89]. Surprisingly, 2'-O-Me focuses on modifying specific regions of mRNA where the encoded amino acids are immobilized; these amino acids include glutamate, lysine and glutamine [90]. This phenomenon hints at the hypothesis that 2'-O-Me has the potential to affect translation efficiency, which has previously been demonstrated in bacterial mRNA [81].

Regulatory roles of RNA modifications in pathogenesis
Aberrant m⁶A RNA modifications in diseases
In acute myeloid leukaemia (AML), FTO decreases m^6A abundance on ASB2 and RARA mRNA in several certain subtypes of AML, including t(11q23)/MLL rearrangements, t(15;17)/PML-RARA, FLT3-ITD, and/or NPM1 mutations [41, 108]. Moreover, by constraining YTHDF2-mediated decay, FTO decreases m^6A frequency on MYC mRNA [109], METTL3 promotes translation of BCL2 and PTEN mRNA by upregulating the m^6A levels and supports expression of SP1 by binding to the unique region with the help of the

Shi *et al. Molecular Cancer*   (2020) 19:78

Page 8 of 17

transcription factor CEBPZ [110, 111], and METTL14 enhances mRNA expression of MYB and MYC [112]. All pathological pathways contribute to carcinogenesis in AML. According to the datasets from The Cancer Genome Atlas, nearly 10.5% of AML patients carry copy number variations (CNVs) of ALKBH5, which predicts poor prognosis and p53 mutations [113].

In gastric cancer (GC), METTL3 can cause m^6A to accumulate on HDGF mRNA, which indicates proliferation and poor prognosis and enhances the stability of zinc finger MYM-type containing 1 (ZMYM1) mRNA so that it accelerates epithelial-mesenchymal transition (EMT) and metastasis [114, 115]. However, METTL3 can also reduce m^6A on SEC62 with the help of MiR-4429 [116]. In hepatocellular carcinoma (HCC), METTL3 enhances the degradation of m^6A-containing SOCS2 mRNA together with YTHDF2 [117]. Additionally, YTHDF2 supresses ERK/MAPK signalling cascades and cell proliferation by destabilizing the EGFR mRNA [118]. Regarding clinical diagnosis, downregulated METTL14 is detected in HCC patients, and the level of expression in metastatic HCC is further decreased [119]. In pancreatic cancer, m^6A and METTL3 protein and mRNA levels were much higher in tumour specimens than in para-cancerous specimens [120]. Meanwhile, upregulation of YTHDF2 destabilizes YAP mRNA by initiating the AKT/GSK3β/cyclin D1 pathway, which promotes proliferation and inhibits the migration of pancreatic cancer [121].

In lung cancer, METTL3 enhances the translation of EGFR and TAZ mRNA [56]. Furthermore, SUMOylated METTL3 promotes non-small-cell lung cancer (NSCLC) by diminishing the amount of m^6A [122]. Moreover, YTHDF2 enhances the translation of 6-phosphogluconate dehydrogenase (6PGD) mRNA by binding to a given region in lung cancer cells [123]. Additionally, FTO is overexpressed in human NSCLC tissues and stimulates lung cancer by stabilizing and increasing the expression of ubiquitin-specific protease 7 (USP7) [124]. In lung squamous cell carcinoma (LUSC), overexpressed FTO accelerates oncogene MZF1 expression by diminishing m^6A and stabilizing mRNA as well [125, 126].

For the nervous system, decreased levels of METTL3 or METTL14 determine the diminution of m^6A on ADAM19 mRNA, which promotes protein expression [127, 128]. Conversely, increased levels of ALKBH5 lead to decreased levels of m^6A on FOXM1 mRNA and enhance protein expression [129]. Consequently, a high level of ALKBH5 predicts poor prognosis [130]. However, both pathways can contribute to glioblastoma. Subsequently, overexpressed METTL3 recruits HuR to modified SOX2 mRNA and enhances radio-resistance. Playing an oncogenic role in glioblastoma, METTL3 hints at poor prognosis and a potential therapeutic strategy as well [131].

In prostate cancer, reduced YTHDF2 elevates m^6A contents dramatically, which suppresses proliferation and migration [132]. In bladder cancer, increased METTL3 predicts poor survival because with the help of pri-miR221/222, upregulated METTL3 results in downregulated PTEN and tumorigenesis of cancer [133].

Aberrant m^6A modification can also lead to carcinomas in the reproductive system. It has been reported that m^6A on KLF4 and NANOG can be suppressed by the cooperation of ZNF217 and ALKBH5, especially in a HIF-dependent manner, so that it enhances the stability of mRNA and contributes to breast cancer in a hypoxic microenvironment [134, 135]. Increased METTL3 leads to enhancement of m^6A on hepatitis B X-interacting protein (HBXIP) and proliferation of breast cancer stem cells (BCSCs) [136]. Moreover, elevated FTO leads to downregulated methylation and degradation of BNIP3. It is suggested that FTO enhances the colony formation and metastasis of breast cancer [137]; Nevertheless, in cervical squamous cell carcinoma (CSCC), high expression of FTO and low levels of β-catenin lead to chemoradiotherapy resistance, which hints that FTO is a potential target to increase the chemoradiotherapy sensitivity of CSCC [138]. In endometrial cancer, either mutated METTL14 or reduced METTL3 limits the expression of m^6A. However, limited m^6A activates the AKT signalling pathway and stimulates proliferation and tumorigenicity by decreasing the negative AKT regulator PHLPP2 and increasing the positive AKT regulator mTORC2 [139].

Besides the regular cancers with high incidence referenced above, aberrant m^6A modifications also play roles in sensory organs. The fate of ocular melanoma can be modulated by m^6A modifications. With the help of YTHDF1, the translation of methylated HINT2 mRNA, a tumour suppressor of ocular melanoma, was significantly accelerated, meaning m^6A modification obviously inhibits the progression of ocular melanoma. Moreover, investigation of ocular melanoma samples indicated that decreased m^6A levels were highly associated with poor prognosis [140].

Aberrant m^1A RNA modification in diseases

Physiological functions lead to pathological impacts on diverse diseases. In ovarian and breast cancers, demethylation of m^1A by ALKBH3 induces increased modified CSF-1 mRNA, which contains m^1A in the 5'UTR near the translation initiation site. Hence, accumulated ALKBH3 means improved CSF-1 mRNA expression and invasion of cancer cells [141]. Subsequently, ALKBH3, considered the eraser of m^1A, tightly correlates with the mTOR pathway in gastrointestinal cancer and is attributed to the limited expression of ErbB2 and AKT1S1 after ALKBH3 knockdown; the downstream genes of

Shi et al. Molecular Cancer   (2020) 19:78

Page 9 of 17

m^{1}A are associated with cell proliferation according to Gene Ontology analysis [142]. Additionally, silencing of ALKBH3 arrests the cell cycle at the G1 phase and contributes to the progression, angiogenesis and invasion of urothelial carcinomas by modulating NADPH oxidase-2-reactive oxygen species (NOX-2-ROX) and TNF-like weak inducer of apoptosis (TWEAK)/Fibroblast growth factor-inducible 14 (Fn14)-VEGF signals [143]. As a classical chemical modification of mRNA, the pathological pathways of m^{1}A need to be elucidated.

Aberrant m^{5}C RNA modification in diseases

Since m^{5}C bridges transcription and translation, we propose a hypothesis that m^{5}C can also regulate the pathological mechanisms of various diseases. For instance, diminishing NSUN2 leads to decreased levels of translation and an increased tumour initiating population in skin cancer [144]. In breast cancer, NSUN2 is reported to be upregulated as well at the mRNA and protein levels [145]. For patients with urothelial carcinoma of the bladder (UCB), m^{5}C-modified 3'UTR in HDGF mRNA can be recognized by YBX1 and activate the oncogene of UCB [78]. m^{5}C can also be regarded as a cancer biomarker because the amount of m^{5}C RNA modification is increased in circulating tumour cells from patients with lung cancer [146].

Aberrant hm^{5}C, Ψ, I, U and 2'-O-Me RNA modifications in diseases

Although the amounts of hm^{5}C, Ψ, I, U and 2'-O-Me RNA modifications on mRNA are much lower than the three predominant types of modifications, their roles do not change and are vital to human disease. First, Ψ can function as a biomarker for prostate cancer because certain nucleolar RNAs (H/ACA snoRNAs) and the dyskerin (DKC1) protein can upregulate the transformation of U to Ψ and contribute to the advancement to cancer [147]. Regarded as the gene encoding the Ψ synthase, the mutation of DKC1 causes downregulated Ψ and X-linked dyskeratosis congenita (X-DC) [148]. The risk for cancer development is higher in patients with X-DC than those without gene mutation [149]. Besides, H/ACA snoRNAs are limited in acute leukaemia, lymphoma and multiple myeloma [150–152].

Subsequently, edited AZIN1 stimulates a serine to glycine (S/G) conversion in HCC and leads to proliferation and poor prognosis [153, 154]. In HCC and in cervical cancer, increased editing of BLCAP activates the AKT/mTOR signalling pathway or STAT3, which can increase cell proliferation and limit apoptosis [155–158]. In breast cancer, editing of DHFR transcripts at the 3'UTR by ADAR1 stabilizes the mRNA and enhances cell growth. Surprisingly, methotrexate, a chemotherapy agent, prevents cancer cell division by targeting DHFR.

It is suggests that downregulated ADAR1 can contribute to methotrexate treatment [159]. In gastric cancer, ADAR2 edits the CDS of PODXL, which induces a histidine to arginine conversion. The relationship between reduced ADAR2 and increased malignancy hints that transcript editing is essential to prevent cancer progression [160]. Additionally, adenosine deaminase RNA-specific B1 (ADARB1), a special type of ADAR, is expressed at low levels in H358 and A549 lung adenocarcinoma (LUAD) cells, which suggests that I might be a potential target in diagnostic and prognostic progression for patients with LUAD [161].

Finally, uridine phosphorylase 1 (UPP1) is another enzyme that can reversibly catalyse the phosphorolysis of uridine to uracil [162, 163]. It has been reported that expression of UPP1 significantly depends on lymph node metastasis and tumour stage and size in patients with thyroid carcinoma [164] (Table 3, Fig. 4).

Clinical prospects of RNA modifications

RNA modifications and enzyme complexes exhibit upregulated and downregulated levels of expression in cancers, which means RNA modifications can serve as biomarkers to diagnose diseases in a manner that is helpful and precise. For example, upregulated YTHDF2 is found in pancreatic cancer, increased m^{5}C is detected in lung cancer and accumulated Ψ contributes to the advancement of prostate cancer [121, 146, 147]. However, other biomarkers need to be elucidated. Besides biomarkers to diagnose cancers, RNA modifications are also biomarkers to predict patient prognosis. Since they stimulate or inhibit the progression of cancer, RNA modifications have therapeutic potential. 3-deazaadenosine (DAA) interrupts METTL3/14 and inhibits the decoration of m^{6}A by obstructing SAH hydrolase [165], SPI1 is considered a potential target for AML because of inhibition of METTL14 [112], and meclofenamic acid (MA), a non-steroidal anti-inflammatory drug, silences FTO by competing for binding sites [166]. Novel targets for treatment of cancer require further investigation.

Conclusion

In summary, chemical modifications in mRNA are vital for many processes of cell life, such as pre-mRNA splicing, nuclear export, transcript stability and translation initiation. Importantly, RNA modifications play a critical role in driving cell fate in cancer. The importance of the relationship between RNA modification and various diseases cannot be overly emphasized. In this review, we redefined the bridge between transcription and translation and applied it to physiological and pathological processes. To date, we have demonstrated 2 roles of mRNA modifications in transcription. Generally, one type is

Table 3 Aberrant mRNA modifications in diseases

Modification	Disease	Enzyme	Target	Description	Ref
m⁶A	AML	FTO	ASB2/RARA	FTO decreases m⁶A abundance on ASB2 and RARA mRNA in certain subtypes of AML and diminishes the amount of protein	[41, 108]
		FTO	MYC	FTO decreases m⁶A frequency on MYC mRNA by limiting YTHDF2-mediated RNA decay	[109]
		METTL3	BCL2/PTEN	METTL3 promotes the translation of BCL2 and PTEN mRNA by upregulating m⁶A levels	[110]
			SP1	METTL3 supports the expression of SP1 by binding to the unique region with the help of the transcription factor CEBPZ	[111]
		METTL14	MYB/MYC	METTL14 enhances the expression of MYB and MYC mRNA in AML	[112]
		ALKBH5	N.A.	Approximately 10.5% of AML patients carry CNVs of ALKBH5, which predicts poor prognosis and p53 mutations	[113]
	Gastric cancer	METTL3	HDGF	METTL3 causes m⁶A to accumulate on HDGF mRNA, which indicates proliferation and poor prognosis of gastric cancer	[114]
			ZMYM1	METTL3 enhances the stability of ZMYM1 mRNA to accelerate EMT and metastasis	[115]
			SEC62	METTL3 reduces m⁶A on SEC62 with the help with MiR-4429	[116]
	Hepatic carcinoma	METTL3	SOCS2	METTL3 works with YTHDF2 together to enhance the degradation of SOCS2 m⁶A-containing mRNA, which leads to HCC	[117]
		YTHDF2	EGFR	YTHDF2 suppresses ERK/MAPK signalling cascades and cell proliferation via destabilizing the EGFR mRNA	[118]
		METTL14	N.A.	The expression of METTL14 is decreased in HCC, especially in metastatic HCC	[119]
	Pancreatic cancer	METTL3	N.A.	METTL3 protein, m⁶A abundance and mRNA levels are much higher in tumour specimens than in para-cancerous specimens	[120]
		YTHDF2	YAP	Increased YTHDF2 promotes proliferation and suppresses migration of pancreatic cancer by destabilizing YAP mRNA	[121]
	Lung cancer	METTL3	EGFR/TAZ	METTL3 enhances the translation of EGFR and TAZ mRNA in lung cancer	[56]
		SUMOylated METTL3	N.A.	SUMOylated METTL3 promotes NSCLC by diminishing the amount of m⁶A	[122]
		YTHDF2	6PGD	YTHDF2 enhances 6PGD mRNA translation by binding to m⁶A sites uniquely in lung cancer cells	[123]
		FTO	USP7	FTO stabilizes and increases the expression of USP7 by reducing m⁶A content	[124]
		FTO	MZF1	Overexpressed FTO accelerates oncogene MZF1 expression by diminishing m6A and stabilizing MZF1 in LUSC	[125, 126]
	Glioblastoma	METTL3/METTL14	ADAM19	Decreased METTL3 or METTL14 determines the diminution of m⁶A on ADAM19 mRNA, which promotes the expression of protein and contributes to glioblastoma	[127, 128]
		ALKBH5	FOXM1	Increased levels of ALKBH5 lead to decreased levels of m⁶A on FOXM1 mRNA and enhance protein translation, which predicts poor prognosis	[129, 130]
		METTL3	SOX2	Elevated METTL3 stabilizes SOX2 mRNA and enhances radio-resistance of glioblastoma	[131]
	Prostate cancer	YTHDF2	N.A.	Downregulated YTHDF2 suppresses the proliferation and migration of prostate cancer by elevating m⁶A contents	[132]
	Bladder cancer	METTL3	PTEN	With the help of pri-miR221/222, upregulated METTL3 leads to downregulated PTEN and tumorigenesis of cancer	[133]
	Breast cancer	ALKBH5	KLF4/NANOG	m⁶A on KLF4 and NANOG can be suppressed by the cooperation of ZNF17 and ALKBH5 to promote protein expression and contribute to breast cancer	[134, 135]
		METTL3	HBXIP	Enhanced levels of m⁶A on HBXIP are attributed to increased METTL3 and promote the proliferation of breast cancer stem cells	[136]
		FTO	BNIP3	Elevated FTO leads to decreased expression of BNIP3 and metastasis of breast cancer	[137]

Shi *et al. Molecular Cancer*   (2020) 19:78

Page 11 of 17

Table 3 Aberrant mRNA modifications in diseases *(Continued)*

Modification	Disease	Enzyme	Target	Description	Ref
	Cervical cancer	FTO	β-catenin	High expression of FTO and low levels of β-catenin lead to chemoradiotherapy resistance in cervical squamous cell carcinoma	[138]
	Endometrial cancer	METTL14/ METTL3	N.A.	Either mutated METTL14 or reduced METTL3 activates the AKT signalling pathway and stimulates proliferation and tumorigenicity by limiting the expression of m^{6}A	[139]
	Ocular melanoma	YTHDF1	HINT2	YTHDF1 promotes the translation of methylated HINT2 mRNA and inhibits the progression of ocular melanoma	[140]
m^{1}A	Ovarian/Breast cancer	ALKBH3	CSF-1	Accumulated ALKBH3 indicates improved CSF-1 mRNA expression and invasion of cancer cells	[141]
	Gastrointestinal cancer	ALKBH3	ErbB2/ AKT1S1	Aberrant m^{1}A modifications regulate gastrointestinal cancer by modulating the mTOR pathway associated with cell proliferation	[142]
	Urothelial carcinoma	ALKBH3	N.A.	ALKBH3 promotes the progression, angiogenesis and invasion of urothelial carcinomas via NOX-2-ROS and TWEAK/Fn14-VEGF signals	[143]
m^{5}C	Skin cancer	NSUN2	N.A.	Inactivating NSUN2 prevents protein translation and stimulates the tumour-initiating population of skin cancer	[144]
	Breast cancer	NSUN2	N.A.	NSUN2 is reported to be upregulated at the mRNA and protein levels	[145]
	Urothelial carcinoma	YBX1	HDGF	m^{5}C modified 3'UTR in HDGF mRNA can be recognized by YBX1 and activate the advancement of UCB	[78]
	Lung cancer	N.A.	N.A.	M^{5}C RNA modification is upregulated in circulating tumour cells from patients with lung cancer	[146]
Ψ	Prostate cancer	DKC1	N.A.	Certain nucleolar RNAs (H/ACA snoRNAs) and DKC1 that transfer U to Ψ contribute to the progression of cancer	[147]
	Haematological malignancies	N.A.	N.A.	H/ACA snoRNAs are limited in acute leukaemia, lymphoma and multiple myeloma	[150–152]
I	Hepatocellular carcinoma	ADAR1	AZIN1	Edited AZIN1 stimulates S/G conversion and induces proliferation and poor prognosis in hepatocellular carcinoma	[153, 154]
		ADAR1	BLCAP	Increased editing of BLCAP accelerates cell proliferation by activating the Akt/mTOR signalling pathway or STAT3	[158]
	Cervical cancer	ADAR1	BLCAP	Increased editing of BLCAP accelerates cell proliferation by activating the Akt/mTOR signalling pathway or STAT3	[157]
	Breast cancer	ADAR1	DHFR	Editing of DHFR by ADAR1 stabilizes mRNA and accelerates cell growth	[159]
	Gastric cancer	ADAR2	PODXL	Downregulated ADAR2 reduces the decoration on PODXL and increases the malignancy of gastric cancer	[160]
	Lung adenocarcinoma	ADARB1	N.A.	ADARB1 has low expression in H358 and A549 lung adenocarcinoma cells	[161]
U	Thyroid carcinoma	UPP1	N.A.	It is reported that the expression of UPP1 significantly depends on lymph node metastasis, tumour stage and size	[164]
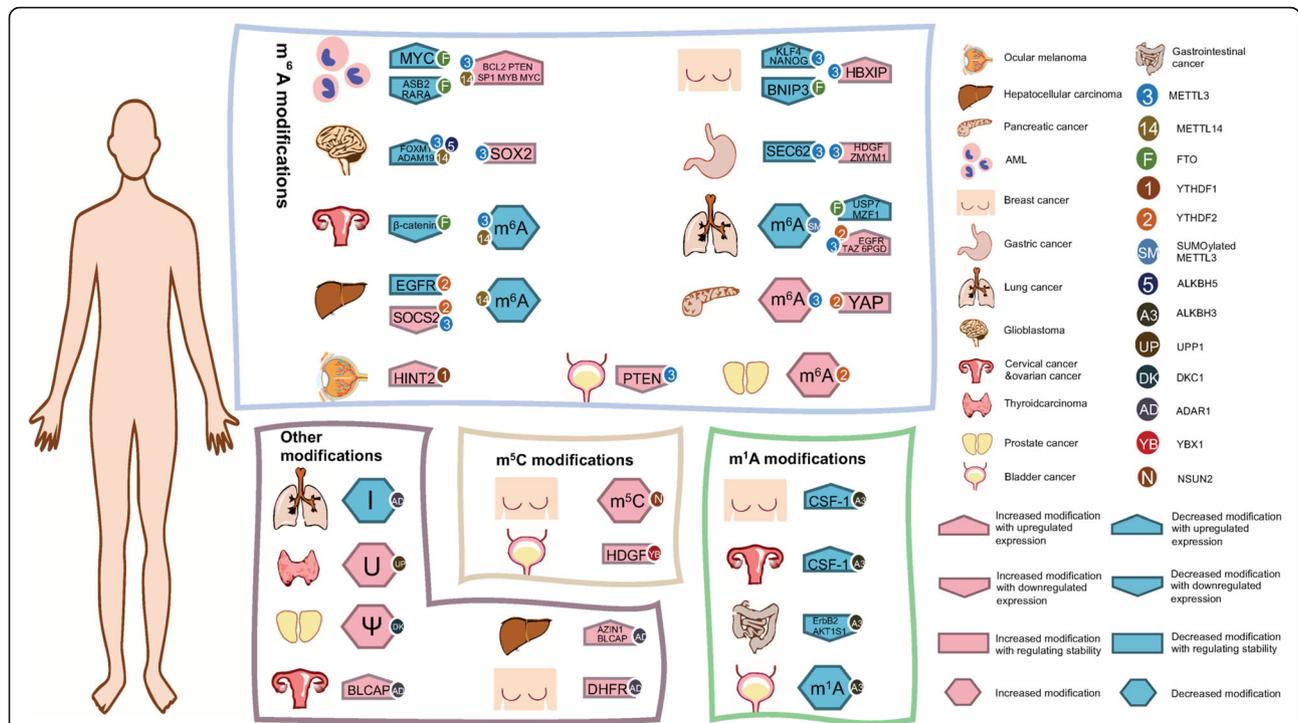
Fig. 4 Regulatory roles of RNA modifications in pathogenesis. Applying physiology to pathology, RNA modifications redefine the bridge between transcription and translation and regulate disease pathogenesis. In AML, METTL3 and METTL14 enhance the expression of m⁶A modifications as well as the BCL2, PTEN, SP1, MYB and MYC genes, which lead to tumour progression. Simultaneously, FTO decreases m⁶A abundance on ASB2 and RARA mRNA. In digestive system tumours, aberrant METTL3 leads to aberrant expression of HDGF, ZMYM1, SEC62 and SOCS2, which can regulate cancer cells in the stomach, liver and pancreas, respectively. In lung cancer, METTL3 enhances the translation of EGFR and TAZ, whereas SUMOylated METTL3 promotes NSCLC; aberrant YTHDF2 enhances the expression of 6PGD in lung cancer, and overexpressed FTO stabilizes and accelerates the expression of USF7 and MZF1 as well. In glioblastoma, METTL3, METTL14 and ALKBH5 promote the expression of ADAM19 and FOXM1 and predict poor prognosis. In prostate cancer, aberrant YTHDF2 suppresses proliferation and migration. In bladder cancer, METTL3 reduces the expression of PTEN and tumorigenesis of cancer. In the reproductive system, METTL3 and FTO contribute to the aberrant expression of KLF4, NANOG, HBXIP, BNIP3 and β-catenin, which induce proliferation of breast cancer and chemoradiotherapy resistance of cervical cancer separately. In sensory organs, YTHDF1 accelerates the translation of methylated HINT2 and inhibits the progression of ocular melanoma. Aberrant eraser ALKBH3 reduces m¹A modifications, leads to aberrant expression of CSF-1, ErbB2 and AKT1S1, and induces the progression of ovarian cancer, breast cancer, gastrointestinal cancer and urothelial cancer. In UCB, YBX1 recognizes m⁵C-modified HDGF mRNA and leads to tumour advancement. Upregulated USUN2 is detected in breast cancer. Ultimately, aberrant ADAR1 edits AZIN1, BLCAP, and DHFR separately, which leads to hepatocellular carcinoma, cervical cancer and breast cancer. Additionally, together with Ψ, I and U, DKC1, ADAR1 and UPP1 can function as biomarkers to indicate prostate cancer progression, LUAD presentation and thyroid carcinoma status

mRNA modifications that can change the structure of transcripts, and the other is mRNA modifications that can regulate transcription by joining hands with a complex of enzymes, such as METTL3 or NSUN2. Considering that modifications can regulate the fate of diverse diseases, such modifications have the potential to be utilized in targeted therapy. Surely, RNA modifications as well as the related diseases mentioned above are a fraction of those affecting human beings in nature. Thus, these modifications need to be elucidated in the following few years.

Shi *et al. Molecular Cancer*   (2020) 19:78

Page 13 of 17

HIF: Hypoxia inducible factor; hm^{5}C: 5-hydroxymethylcytosine;
HNRNP: Heterogeneous nuclear ribonucleoprotein; HuR: Hu antigen R;
IFIT: IFN-induced proteins with tetratricopeptide repeats; IGF2BP: Insulin-like
growth factor 2 mRNA-binding protein; IL-17A: Interleukin-17A; IRE: Iron-
responsive element; KLF4: Kruppel like factor 4; LRPPRC: Leucine-rich
pentatricopeptide repeat-containing; LUAD: Lung adenocarcinoma;
LUSC: Lung squamous cell carcinoma; m^{1}A: N1-methyladenosine; m^{6}A: N6-
methyladenosine; m^{5}C: 5-methylcytosine; MA: Meclofenamic acid;
MAPK: Mitogen-activated protein kinase; mESC: Mouse embryonic stem cell;
METTL3: Methyltransferase-like 3; METTL14: Methyltransferase-like14; MiR-
4429: MicroRNA 4429; MLL: Mixed lineage leukaemia; mRNA: Message RNA;
mt-mRNA: Mitochondrial mRNA; mTOR: Mammalian target of rapamycin;
MYB: Myeloblastosis oncogene; MYC: Myelocytomatosis oncogene;
MZF1: Myeloid zinc finger 1; NANOG: Nanog homeobox;
ND5: NADHubiquinone oxidoreductase core subunit 5; NOX-2-ROX: NADPH
oxidase-2-reactive oxygen species; NPC: Neplanocin A;
NPM1: Nucleophosmin 1; NSCLC: Non-small-cell lung cancer; NSUN: NOL1/
NOP2/SUN domain; NXF1: Nuclear RNA export factor 1; Per2: Period circadian
regulator 2; PHLPP2: PH domain and leucine rich repeat protein phosphatase
2; PML: Promyelocytic leukaemia; PODXL: Podocalyxin like;
PTEN: Phosphatase and tensin homologue; Q: Queuosine; RA: Rheumatoid
arthritis; RARA: Retinoic acid receptor alpha; RBM15: RNA-binding motif
protein 15; rRNA: Ribosomal RNA; SAH: S- adenosylhomocysteine;
SEC62: SEC62 homologue, preprotein translocation factor; snRNA: Small
nuclearRNA; SOCS2: Suppressor of cytokine signalling 2; SOX2: SRY-box
transcription factor 2; SP1: Sp1 transcription factor; SPI1: Spi-1 proto-
oncogene; SRSF3: Serine and arginine rich splicing factor 3; STAT3: Signal
transducer and activator of transcription 3; SUMO: Small ubiquitin-like modi-
fier; T2DM: Type 2 diabetes mellitus; TAZ: Tafazzin; TRMT: tRNA
methyltransferase; tRNA: Transfer RNA; TWEAK: TNF-like weak inducer of
apoptosis; U2AF: U2 auxiliary factor; UCB: Urothelial carcinoma of the
bladder; UPP1: Uridine phosphorylase 1; USP7: ubiquitin-specific protease 7;
VEGF: Vascular endothelial growth factor; WTAP: Wilms tumour 1-associated
protein; X-DC: X-linked dyskeratosis congenita; YAP: Yes-associated protein;
YBX1: Y-Box binding protein 1; YTH: YT521-B homology; ZC3H13: Zinc finger
CCCH-type containing 13; ZMYM1: Zinc finger MYM-type containing 1;
ZNF217: Zinc finger protein 217

Acknowledgements
Not applicable

Authors' contributions
RJ and XF provided direction and guidance throughout the preparation of
this manuscript. HS collected and interpreted studies and was a major
contributor to the writing and editing of the manuscript. PC reviewed and
made significant revisions to the manuscript. All authors read and approved
the final manuscript.

Availability of data and materials
Not applicable

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Ophthalmology, Ninth People's Hospital, Shanghai JiaoTong
University School of Medicine, Shanghai 20025, P.R. China. [2]Shanghai Key
Laboratory of Orbital Diseases and Ocular Oncology, Shanghai 20025,
People's Republic of China.

References
1. Davis FF, Allen FW. Ribonucleic acids from yeast which contain a fifth
   nucleotide. J Biol Chem. 1957;227:907–15.
2. Wei CM, Gershowitz A, Moss B. Methylated nucleotides block 5' terminus of
   HeLa cell messenger RNA. Cell. 1975;4:379–86.
3. Cohn WE. Pseudouridine, a carbon-carbon linked ribonucleoside in
   ribonucleic acids: isolation, structure, and chemical characteristics. J Biol
   Chem. 1960;235:1488–98.
4. Dubin DT, Taylor RH. The methylation state of poly A-containing messenger
   RNA from cultured hamster cells. Nucleic Acids Res. 1975;2:1653–68.
5. Desrosiers R, Friderici K, Rottman F. Identification of methylated nucleosides
   in messenger RNA from Novikoff hepatoma cells. Proc Natl Acad Sci U S A.
   1974;71:3971–5.
6. Juhling F, Morl M, Hartmann RK, Sprinzl M, Stadler PF, Putz J. tRNAdb 2009:
   compilation of tRNA sequences and tRNA genes. Nucleic Acids Res. 2009;37:
   D159–62.
7. Roundtree IA, Evans ME, Pan T, He C. Dynamic RNA modifications in gene
   expression regulation. Cell. 2017;169:1187–200.
8. Alarcon CR, Goodarzi H, Lee H, Liu X, Tavazoie S, Tavazoie SF. HNRNPA2B1 is
   a mediator of m(6)A-dependent nuclear RNA processing events. Cell. 2015;
   162:1299–308.
9. Alarcon CR, Lee H, Goodarzi H, Halberg N, Tavazoie SF. N6-methyladenosine
   marks primary microRNAs for processing. Nature. 2015;519:482–5.
10. Liu N, Dai Q, Zheng G, He C, Parisien M, Pan T. N(6)-methyladenosine-
    dependent RNA structural switches regulate RNA-protein interactions.
    Nature. 2015;518:560–4.
11. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, Vagbo CB, Shi Y,
    Wang WL, Song SH, et al. ALKBH5 is a mammalian RNA demethylase that
    impacts RNA metabolism and mouse fertility. Mol Cell. 2013;49:18–29.
12. Wang X, Lu Z, Gomez A, Hon GC, Yue Y, Han D, Fu Y, Parisien M, Dai Q, Jia
    G, et al. N6-methyladenosine-dependent regulation of messenger RNA
    stability. Nature. 2014;505:117–20.
13. Bjork GR, Hagervall TG. Transfer RNA modification: presence, synthesis, and
    function. EcoSal Plus. 2014;6.
14. Rezgui VA, Tyagi K, Ranjan N, Konevega AL, Mittelstaet J, Rodnina MV, Peter
    M, Pedrioli PG. tRNA tKUUU, tQUUG, and tEUUC wobble position
    modifications fine-tune protein translation by promoting ribosome A-site
    binding. Proc Natl Acad Sci U S A. 2013;110:12289–94.
15. Tukenmez H, Xu H, Esberg A, Bystrom AS. The role of wobble uridine
    modifications in +1 translational frameshifting in eukaryotes. Nucleic Acids
    Res. 2015;43:9489–99.
16. Hori H. Methylated nucleosides in tRNA and tRNA methyltransferases. Front
    Genet. 2014;5:144.
17. Gustilo EM, Vendeix FA, Agris PF. tRNA's modifications bring order to gene
    expression. Curr Opin Microbiol. 2008;11:134–40.
18. Delaunay S, Frye M. RNA modifications regulating cell fate in cancer. Nat
    Cell Biol. 2019;21:552–9.
19. Krug RM, Morgan MA, Shatkin AJ. Influenza viral mRNA contains internal N6-
    methyladenosine and 5'-terminal 7-methylguanosine in cap structures. J
    Virol. 1976;20:45–53.
20. Sommer S, Salditt-Georgieff M, Bachenheimer S, Darnell JE, Furuichi Y,
    Morgan M, Shatkin AJ. The methylation of adenovirus-specific nuclear and
    cytoplasmic RNA. Nucleic Acids Res. 1976;3:749–65.
21. Kennedy TD, Lane BG. Wheat embryo ribonucleates. XIII. Methyl-substituted
    nucleoside constituents and 5'-terminal dinucleotide sequences in bulk
    poly(AR)-rich RNA from imbibing wheat embryos. Can J Biochem. 1979;57:
    927–31.
22. Deng X, Chen K, Luo GZ, Weng X, Ji Q, Zhou T, He C. Widespread
    occurrence of N6-methyladenosine in bacterial mRNA. Nucleic Acids Res.
    2015;43:6557–67.
23. Adams JM, Cory S. Modified nucleosides and bizarre 5'-termini in mouse
    myeloma mRNA. Nature. 1975;255:28–33.

Shi *et al. Molecular Cancer*   (2020) 19:78

Page 14 of 17

24. Kane SE, Beemon K. Precise localization of m6A in Rous sarcoma virus RNA reveals clustering of methylation sites: implications for RNA processing. Mol Cell Biol. 1985;5:2298–306.

25. 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.

26. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature. 2012;485:201–6.

27. Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, Pestova TV, Qian SB, Jaffrey SR. 5' UTR m(6)A promotes cap-independent translation. Cell. 2015;163:999–1010.

28. Zhou J, Wan J, Gao X, Zhang X, Jaffrey SR, Qian SB. Dynamic m(6)A mRNA methylation directs translational control of heat shock response. Nature. 2015;526:591–4.

29. Bokar JA, Shambaugh ME, Polayes D, Matera AG, Rottman FM. Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. RNA. 1997;3:1233–47.

30. Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, Jia G, Yu M, Lu Z, Deng X, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat Chem Biol. 2014;10:93–5.

31. Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, Mertins P, Ter-Ovanesyan D, Habib N, Cacchiarelli D, et al. Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. Cell Rep. 2014;8:284–96.

32. Visvanathan A, Somasundaram K. mRNA traffic control reviewed: N6-Methyladenosine (m(6) A) takes the driver's seat. Bioessays. 2018;40.

33. Wen J, Lv R, Ma H, Shen H, He C, Wang J, Jiao F, Liu H, Yang P, Tan L, et al. Zc3h13 regulates nuclear RNA m6A methylation and mouse embryonic stem cell self-renewal. Mol Cell. 2018;69:1028–38 e1026.

34. Luo S, Tong L. Molecular basis for the recognition of methylated adenines in RNA by the eukaryotic YTH domain. Proc Natl Acad Sci U S A. 2014;111: 13834–9.

35. Xu C, Wang X, Liu K, Roundtree IA, Tempel W, Li Y, Lu Z, He C, Min J. Structural basis for selective binding of m6A RNA by the YTHDC1 YTH domain. Nat Chem Biol. 2014;10:927–9.

36. Zhu T, Roundtree IA, Wang P, Wang X, Wang L, Sun C, Tian Y, Li J, He C, Xu Y. Crystal structure of the YTH domain of YTHDF2 reveals mechanism for recognition of N6-methyladenosine. Cell Res. 2014;24:1493–6.

37. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, Weng X, Chen K, Shi H, He C. N(6)-methyladenosine modulates messenger RNA translation efficiency. Cell. 2015;161:1388–99.

38. Xiao W, Adhikari S, Dahal U, Chen YS, Hao YJ, Sun BF, Sun HY, Li A, Ping XL, Lai WY, et al. Nuclear m(6)A reader YTHDC1 regulates mRNA splicing. Mol Cell. 2016;61:507–19.

39. 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;20:285–95.

40. Arguello AE, DeLiberto AN, Kleiner RE. RNA chemical proteomics reveals the N(6)-Methyladenosine (m(6)A)-regulated protein-RNA interactome. J Am Chem Soc. 2017;139:17249–52.

41. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG, He C. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol. 2011;7:885–7.

42. Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, Hewitson KS, Yeo GS, McDonough MA, Cunliffe S, McNeill LA, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science. 2007;318:1469–72.

43. Stoltzfus CM, Dane RW. Accumulation of spliced avian retrovirus mRNA is inhibited in S-adenosylmethionine-depleted chicken embryo fibroblasts. J Virol. 1982;42:918–31.

44. Finkel D, Groner Y. Methylations of adenosine residues (m6A) in pre-mRNA are important for formation of late simian virus 40 mRNAs. Virology. 1983; 131:409–25.

45. Caboche M, Bachellerie JP. RNA methylation and control of eukaryotic RNA biosynthesis. Effects of cycloleucine, a specific inhibitor of methylation, on ribosomal RNA maturation. Eur J Biochem. 1977;74:19–29.

46. Hayashi M, Yaginuma S, Yoshioka H, Nakatsu K. Studies on neplanocin A, new antitumor antibiotic. ll. Structure determination. J Antibiot (Tokyo). 1981;34:675–80.

47. Zhou KI, Shi H, Lyu R, Wylder AC, Matuszek Z, Pan JN, He C, Parisien M, Pan T. Regulation of Co-transcriptional Pre-mRNA splicing by m(6)A through the low-complexity protein hnRNPG. Mol Cell. 2019;76:70–81 e79.

48. Bartosovic M, Molares HC, Gregorova P, Hrossova D, Kudla G, Vanacova S. N6-methyladenosine demethylase FTO targets pre-mRNAs and regulates alternative splicing and 3'-end processing. Nucleic Acids Res. 2017;45: 11356–70.

49. Fustin JM, Doi M, Yamaguchi Y, Hida H, Nishimura S, Yoshida M, Isagawa T, Morioka MS, Kakeya H, Manabe I, Okamura H. RNA-methylation-dependent RNA processing controls the speed of the circadian clock. Cell. 2013;155: 793–806.

50. Roundtree IA, Luo GZ, Zhang Z, Wang X, Zhou T, Cui Y, Sha J, Huang X, Guerrero L, Xie P, et al. YTHDC1 mediates nuclear export of N(6)-methyladenosine methylated mRNAs. Elife. 2017;6.

51. Wickens M, Bernstein DS, Kimble J, Parker R. A PUF family portrait: 3'UTR regulation as a way of life. Trends Genet. 2002;18:150–7.

52. Wang Y, Li Y, Toth JI, Petroski MD, Zhang Z, Zhao JC. N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nat Cell Biol. 2014;16:191–8.

53. Brennan CM, Steitz JA. HuR and mRNA stability. Cell Mol Life Sci. 2001;58: 266–77.

54. Li A, Chen YS, Ping XL, Yang X, Xiao W, Yang Y, Sun HY, Zhu Q, Baidya P, Wang X, et al. Cytoplasmic m(6)A reader YTHDF3 promotes mRNA translation. Cell Res. 2017;27:444–7.

55. Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsu PJ, Liu C, He C. YTHDF3 facilitates translation and decay of N(6)-methyladenosine-modified RNA. Cell Res. 2017;27:315–28.

56. 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;62:335–45.

57. 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 naive pluripotency toward differentiation. Science. 2015;347:1002–6.

58. Dunn DB. The occurrence of 1-methyladenine in ribonucleic acid. Biochim Biophys Acta. 1961;46:198–200.

59. Machnicka MA, Milanowska K, Osman Oglou O, Purta E, Kurkowska M, Olchowik A, Januszewski W, Kalinowski S, Dunin-Horkawicz S, Rother KM, et al. MODOMICS: a database of RNA modification pathways--2013 update. Nucleic Acids Res. 2013;41:D262–7.

60. Ozanick S, Krecic A, Andersland J, Anderson JT. The bipartite structure of the tRNA m1A58 methyltransferase from S. cerevisiae is conserved in humans. RNA. 2005;11:1281–90.

61. Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, Peer E, Kol N, Ben-Haim MS, Dai Q, Di Segni A, Salmon-Divon M, Clark WC, et al. The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA. Nature. 2016;530:441–6.

62. Li X, Xiong X, Wang K, Wang L, Shu X, Ma S, Yi C. Transcriptome-wide mapping reveals reversible and dynamic N(1)-methyladenosine methylome. Nat Chem Biol. 2016;12:311–6.

63. El Yacoubi B, Bailly M, de Crecy-Lagard V. Biosynthesis and function of posttranscriptional modifications of transfer RNAs. Annu Rev Genet. 2012;46: 69–95.

64. Sharma S, Watzinger P, Kotter P, Entian KD. Identification of a novel methyltransferase, Bmt2, responsible for the N-1-methyl-adenosine base modification of 25S rRNA in Saccharomyces cerevisiae. Nucleic Acids Res. 2013;41:5428–43.

65. Li X, Xiong X, Zhang M, Wang K, Chen Y, Zhou J, Mao Y, Lv J, Yi D, Chen XW, et al. Base-resolution mapping reveals distinct m(1)A methylome in nuclear- and mitochondrial-encoded transcripts. Mol Cell. 2017;68:993–1005 e1009.

66. Safra M, Sas-Chen A, Nir R, Winkler R, Nachshon A, Bar-Yaacov D, Erlacher M, Rossmanith W, Stern-Ginossar N, Schwartz S. The m1A landscape on cytosolic and mitochondrial mRNA at single-base resolution. Nature. 2017; 551:251–5.

67. Dai X, Wang T, Gonzalez G, Wang Y. Identification of YTH domain-containing proteins as the readers for N1-methyladenosine in RNA. Anal Chem. 2018;90:6380–4.

68. Liu F, Clark W, Luo G, Wang X, Fu Y, Wei J, Wang X, Hao Z, Dai Q, Zheng G, et al. ALKBH1-mediated tRNA demethylation regulates translation. Cell. 2016;167:1897.

69. Delatte B, Wang F, Ngoc LV, Collignon E, Bonvin E, Deplus R, Calonne E, Hassabi B, Putmans P, Awe S, et al. RNA biochemistry. Transcriptome-wide distribution and function of RNA hydroxymethylcytosine. Science. 2016;351:282–5.
70. Doerfler W. DNA methylation--a regulatory signal in eukaryotic gene expression. J Gen Virol. 1981;57:1–20.
71. Adams RL, Burdon RH. DNA methylation in eukaryotes. CRC Crit Rev Biochem. 1982;13:349–84.
72. Squires JE, Patel HR, Nousch M, Sibbritt T, Humphreys DT, Parker BJ, Suter CM, Preiss T. Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA. Nucleic Acids Res. 2012;40:5023–33.
73. Xing J, Yi J, Cai X, Tang H, Liu Z, Zhang X, Martindale JL, Yang X, Jiang B, Gorospe M, Wang W. NSun2 promotes cell growth via elevating cyclin-dependent kinase 1 translation. Mol Cell Biol. 2015;35:4043–52.
74. Reid R, Greene PJ, Santi DV. Exposition of a family of RNA m(5)C methyltransferases from searching genomic and proteomic sequences. Nucleic Acids Res. 1999;27:3138–45.
75. Goll MG, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang X, Golic KG, Jacobsen SE, Bestor TH. Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. Science. 2006;311:395–8.
76. Schaefer M, Pollex T, Hanna K, Tuorto F, Meusburger M, Helm M, Lyko F. RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. Genes Dev. 2010;24:1590–5.
77. Yang X, Yang Y, Sun BF, Chen YS, Xu JW, Lai WY, Li A, Wang X, Bhattarai DP, Xiao W, et al. 5-methylcytosine promotes mRNA export - NSUN2 as the methyltransferase and ALYREF as an m(5)C reader. Cell Res. 2017;27:606–25.
78. Chen X, Li A, Sun BF, Yang Y, Han YN, Yuan X, Chen RX, Wei WS, Liu Y, Gao CC, et al. 5-methylcytosine promotes pathogenesis of bladder cancer through stabilizing mRNAs. Nat Cell Biol. 2019;21:978–90.
79. Tang H, Fan X, Xing J, Liu Z, Jiang B, Dou Y, Gorospe M, Wang W. NSun2 delays replicative senescence by repressing p27 (KIP1) translation and elevating CDK1 translation. Aging (Albany NY). 2015;7:1143–58.
80. Li Q, Li X, Tang H, Jiang B, Dou Y, Gorospe M, Wang W. NSUN2-mediated m5C methylation and METTL3/METTL14-mediated m6A methylation cooperatively enhance p21 translation. J Cell Biochem. 2017;118:2587–98.
81. Hoernes TP, Clementi N, Faserl K, Glasner H, Breuker K, Lindner H, Huttenhofer A, Erlacher MD. Nucleotide modifications within bacterial messenger RNAs regulate their translation and are able to rewire the genetic code. Nucleic Acids Res. 2016;44:852–62.
82. Luo Y, Feng J, Xu Q, Wang W, Wang X. NSun2 deficiency protects endothelium from inflammation via mRNA methylation of ICAM-1. Circ Res. 2016;118:944–56.
83. Chen C, Zhao X, Kierzek R, Yu YT. A flexible RNA backbone within the polypyrimidine tract is required for U2AF65 binding and pre-mRNA splicing in vivo. Mol Cell Biol. 2010;30:4108–19.
84. Schwartz S, Bernstein DA, Mumbach MR, Jovanovic M, Herbst RH, Leon-Ricardo BX, Engreitz JM, Guttman M, Satija R, Lander ES, et al. Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. Cell. 2014;159:148–62.
85. Kariko K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, Weissman D. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther. 2008;16:1833–40.
86. Nigita G, Veneziano D, Ferro A. A-to-I RNA editing: current knowledge sources and computational approaches with special emphasis on non-coding RNA molecules. Front Bioeng Biotechnol. 2015;3:37.
87. Licht K, Hartl M, Amman F, Anrather D, Janisiw MP, Jantsch MF. Inosine induces context-dependent recoding and translational stalling. Nucleic Acids Res. 2019;47:3–14.
88. Blanc V, Park E, Schaefer S, Miller M, Lin Y, Kennedy S, Billing AM, Ben Hamidane H, Graumann J, Mortazavi A, et al. Genome-wide identification and functional analysis of Apobec-1-mediated C-to-U RNA editing in mouse small intestine and liver. Genome Biol. 2014;15:R79.
89. Daffis S, Szretter KJ, Schriewer J, Li J, Youn S, Errett J, Lin TY, Schneller S, Zust R, Dong H, et al. 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. Nature. 2010;468:452–6.
90. Dai Q, Moshitch-Moshkovitz S, Han D, Kol N, Amariglio N, Rechavi G, Dominissini D, He C. Nm-seq maps 2'-O-methylation sites in human mRNA with base precision. Nat Methods. 2017;14:695–8.
91. Huber SM, van Delft P, Mendil L, Bachman M, Smollett K, Werner F, Miska EA, Balasubramanian S. Formation and abundance of 5-hydroxymethylcytosine in RNA. Chembiochem. 2015;16:752–5.
92. Fu L, Guerrero CR, Zhong N, Amato NJ, Liu Y, Liu S, Cai Q, Ji D, Jin SG, Niedernhofer LJ, et al. Tet-mediated formation of 5-hydroxymethylcytosine in RNA. J Am Chem Soc. 2014;136:11582–5.
93. Zhang HY, Xiong J, Qi BL, Feng YQ, Yuan BF. The existence of 5-hydroxymethylcytosine and 5-formylcytosine in both DNA and RNA in mammals. Chem Commun (Camb). 2016;52:737–40.
94. Horowitz S, Horowitz A, Nilsen TW, Munns TW, Rottman FM. Mapping of N6-methyladenosine residues in bovine prolactin mRNA. Proc Natl Acad Sci U S A. 1984;81:5667–71.
95. Kane SE, Beemon K. Inhibition of methylation at two internal N6-methyladenosine sites caused by GAC to GAU mutations. J Biol Chem. 1987;262:3422–7.
96. Carlile TM, Rojas-Duran MF, Zinshteyn B, Shin H, Bartoli KM, Gilbert WV. Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. Nature. 2014;515:143–6.
97. Li X, Zhu P, Ma S, Song J, Bai J, Sun F, Yi C. Chemical pulldown reveals dynamic pseudouridylation of the mammalian transcriptome. Nat Chem Biol. 2015;11:592–7.
98. Lovejoy AF, Riordan DP, Brown PO. Transcriptome-wide mapping of pseudouridines: pseudouridine synthases modify specific mRNAs in S. cerevisiae. PLoS One. 2014;9:e110799.
99. Fernandez IS, Ng CL, Kelley AC, Wu G, Yu YT, Ramakrishnan V. Unusual base pairing during the decoding of a stop codon by the ribosome. Nature. 2013;500:107–10.
100. Karijolich J, Yu YT. Converting nonsense codons into sense codons by targeted pseudouridylation. Nature. 2011;474:395–8.
101. Nishikura K. A-to-I editing of coding and non-coding RNAs by ADARs. Nat Rev Mol Cell Biol. 2016;17:83–96.
102. Licht K, Jantsch MF. Rapid and dynamic transcriptome regulation by RNA editing and RNA modifications. J Cell Biol. 2016;213:15–22.
103. Bass BL. RNA editing by adenosine deaminases that act on RNA. Annu Rev Biochem. 2002;71:817–46.
104. Tajaddod M, Jantsch MF, Licht K. The dynamic epitranscriptome: A to I editing modulates genetic information. Chromosoma. 2016;125:51–63.
105. Picardi E, Manzari C, Mastropasqua F, Aiello I, D'Erchia AM, Pesole G. Profiling RNA editing in human tissues: towards the inosinome Atlas. Sci Rep. 2015;5:14941.
106. Lehmann KA, Bass BL. Double-stranded RNA adenosine deaminases ADAR1 and ADAR2 have overlapping specificities. Biochemistry. 2000;39:12875–84.
107. Rosenberg BR, Hamilton CE, Mwangi MM, Dewell S, Papavasiliou FN. Transcriptome-wide sequencing reveals numerous APOBEC1 mRNA-editing targets in transcript 3' UTRs. Nat Struct Mol Biol. 2011;18:230–6.
108. Li Z, Weng H, Su R, Weng X, Zuo Z, Li C, Huang H, Nachtergaele S, Dong L, Hu C, et al. FTO plays an oncogenic role in acute myeloid leukemia as a N(6)-methyladenosine RNA demethylase. Cancer cell. 2017;31:127–41.
109. Su R, Dong L, Li C, Nachtergaele S, Wunderlich M, Qing Y, Deng X, Wang Y, Weng X, Hu C, et al. R-2HG exhibits anti-tumor activity by targeting FTO/m(6)A/MYC/CEBPA signaling. Cell. 2018;172:90–105 e123.
110. 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;23:1369–76.
111. Barbieri I, Tzelepis K, Pandolfini L, Shi J, Millan-Zambrano G, Robson SC, Aspris D, Migliori V, Bannister AJ, Han N, et al. Promoter-bound METTL3 maintains myeloid leukaemia by m(6)A-dependent translation control. Nature. 2017;552:126–31.
112. Weng H, Huang H, Wu H, Qin X, Zhao BS, Dong L, Shi H, Skibbe J, Shen C, Hu C, et al. METTL14 inhibits hematopoietic stem/progenitor differentiation and promotes leukemogenesis via mRNA m(6)A modification. Cell Stem Cell. 2018;22:191–205 e199.
113. Kwok CT, Marshall AD, Rasko JE, Wong JJ. Genetic alterations of m(6)A regulators predict poorer survival in acute myeloid leukemia. J Hematol Oncol. 2017;10:39.
114. Wang Q, Chen C, Ding Q, Zhao Y, Wang Z, Chen J, Jiang Z, Zhang Y, Xu G, Zhang J, et al. METTL3-mediated m(6)A modification of HDGF mRNA promotes gastric cancer progression and has prognostic significance. Gut. 2019.
115. 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.

Shi *et al. Molecular Cancer*   (2020) 19:78

Page 16 of 17

116. He H, Wu W, Sun Z, Chai L. MiR-4429 prevented gastric cancer progression through targeting METTL3 to inhibit m(6)A-caused stabilization of SEC62. Biochem Biophys Res Commun. 2019;517:581–7.

117. 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;67:2254–70.

118. Zhong L, Liao D, Zhang M, Zeng C, Li X, Zhang R, Ma H, Kang T. YTHDF2 suppresses cell proliferation and growth via destabilizing the EGFR mRNA in hepatocellular carcinoma. Cancer Lett. 2019;442:252–61.

119. Ma JZ, Yang F, Zhou CC, Liu F, Yuan JH, Wang F, Wang TT, Xu QG, Zhou WP, Sun SH. METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N(6) -methyladenosine-dependent primary MicroRNA processing. Hepatology. 2017;65:529–43.

120. Xia T, Wu X, Cao M, Zhang P, Shi G, Zhang J, Lu Z, Wu P, Cai B, Miao Y, Jiang K. The RNA m6A methyltransferase METTL3 promotes pancreatic cancer cell proliferation and invasion. Pathol Res Pract. 2019;215:152666.

121. Chen J, Sun Y, Xu X, Wang D, He J, Zhou H, Lu Y, Zeng J, Du F, Gong A, Xu M. YTH domain family 2 orchestrates epithelial-mesenchymal transition/ proliferation dichotomy in pancreatic cancer cells. Cell Cycle. 2017;16:2259–71.

122. Du Y, Hou G, Zhang H, Dou J, He J, Guo Y, Li L, Chen R, Wang Y, Deng R, et al. SUMOylation of the m6A-RNA methyltransferase METTL3 modulates its function. Nucleic Acids Res. 2018;46:5195–208.

123. Sheng H, Li Z, Su S, Sun W, Zhang X, Li L, Li J, Liu S, Lu B, Zhang S, Shan C. YTH domain family 2 promotes lung cancer cell growth by facilitating 6-phosphogluconate dehydrogenase mRNA translation. Carcinogenesis. 2019.

124. Li J, Han Y, Zhang H, Qian Z, Jia W, Gao Y, Zheng H, Li B. The m6A demethylase FTO promotes the growth of lung cancer cells by regulating the m6A level of USP7 mRNA. Biochem Biophys Res Commun. 2019;512: 479–85.

125. Liu J, Ren D, Du Z, Wang H, Zhang H. Jin Y: m(6)A demethylase FTO facilitates tumor progression in lung squamous cell carcinoma by regulating MZF1 expression. Biochem Biophys Res Commun. 2018;502:456–64.

126. Cayir A, Barrow TM, Guo L, Byun HM. Exposure to environmental toxicants reduces global N6-methyladenosine RNA methylation and alters expression of RNA methylation modulator genes. Environ Res. 2019;175:228–34.

127. Cui Q, Shi H, Ye P, Li L, Qu Q, Sun G, Sun G, Lu Z, Huang Y, Yang CG, et al. m(6)A RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. Cell Rep. 2017;18:2622–34.

128. Visvanathan A, Patil V, Abdulla S, Hoheisel JD, Somasundaram K. N(6)-methyladenosine landscape of glioma stem-like cells: METTL3 is essential for the expression of actively transcribed genes and sustenance of the oncogenic signaling. Genes (Basel). 2019;10:141.

129. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, Chen Y, Sulman EP, Xie K, Bogler O, et al. m(6)A demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program. Cancer cell. 2017;31:591–606 e596.

130. Dixit D, Xie Q, Rich JN, Zhao JC. Messenger RNA methylation regulates glioblastoma tumorigenesis. Cancer cell. 2017;31:474–5.

131. Visvanathan A, Patil V, Arora A, Hegde AS, Arivazhagan A, Santosh V, Somasundaram K. Essential role of METTL3-mediated m(6)A modification in glioma stem-like cells maintenance and radioresistance. Oncogene. 2018;37: 522–33.

132. Li J, Meng S, Xu M, Wang S, He L, Xu X, Wang X, Xie L. Downregulation of N(6)-methyladenosine binding YTHDF2 protein mediated by miR-493-3p suppresses prostate cancer by elevating N(6)-methyladenosine levels. Oncotarget. 2018;9:3752–64.

133. Han J, Wang JZ, Yang X, Yu H, Zhou R, Lu HC, Yuan WB, Lu JC, Zhou ZJ, Lu Q, et al. METTL3 promote tumor proliferation of bladder cancer by accelerating pri-miR221/222 maturation in m6a-dependent manner. Mol Cancer. 2019;18:110.

134. Zhang C, Zhi WI, Lu H, Samanta D, Chen I, Gabrielson E, Semenza GL. Hypoxia-inducible factors regulate pluripotency factor expression by ZNF217- and ALKBH5-mediated modulation of RNA methylation in breast cancer cells. Oncotarget. 2016;7:64527–42.

135. Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, He X, Semenza GL. 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.

136. 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

137. breast cancer via inhibiting tumor suppressor let-7g. Cancer Lett. 2018;415: 11–9.

137. Niu Y, Lin Z, Wan A, Chen H, Liang H, Sun L, Wang Y, Li X, Xiong XF, Wei B, et al. RNA N6-methyladenosine demethylase FTO promotes breast tumor progression through inhibiting BNIP3. Mol Cancer. 2019;18:46.

138. Zhou S, Bai ZL, Xia D, Zhao ZJ, Zhao R, Wang YY, Zhe H. FTO regulates the chemo-radiotherapy resistance of cervical squamous cell carcinoma (CSCC) by targeting beta-catenin through mRNA demethylation. Mol Carcinog. 2018;57:590–7.

139. 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;20:1074–83.

140. Jia R, Chai P, Wang S, Sun B, Xu Y, Yang Y, Ge S, Jia R, Yang YG, Fan X. m(6)A modification suppresses ocular melanoma through modulating HINT2 mRNA translation. Mol Cancer. 2019;18:161.

141. Woo HH, Chambers SK. Human ALKBH3-induced m(1)A demethylation increases the CSF-1 mRNA stability in breast and ovarian cancer cells. Biochim Biophys Acta Gene Regul Mech. 2019;1862:35–46.

142. Zhao Y, Zhao Q, Kaboli PJ, Shen J, Li M, Wu X, Yin J, Zhang H, Wu Y, Lin L, et al. m1A regulated genes modulate PI3K/AKT/mTOR and ErbB pathways in gastrointestinal cancer. Transl Oncol. 2019;12:1323–33.

143. Shimada K, Fujii T, Tsujikawa K, Anai S, Fujimoto K, Konishi N. ALKBH3 contributes to survival and angiogenesis of human urothelial carcinoma cells through NADPH oxidase and tweak/Fn14/VEGF signals. Clin Cancer Res. 2012;18:5247–55.

144. Blanco S, Bandiera R, Popis M, Hussain S, Lombard P, Aleksic J, Sajini A, Tanna H, Cortes-Garrido R, Gkatza N, et al. Stem cell function and stress response are controlled by protein synthesis. Nature. 2016;534:335–40.

145. Frye M, Dragoni I, Chin SF, Spiteri I, Kurowski A, Provenzano E, Green A, Ellis IO, Grimmer D, Teschendorff A, et al. Genomic gain of 5p15 leads to over-expression of Misu (NSUN2) in breast cancer. Cancer Lett. 2010;289:71–80.

146. Huang W, Qi CB, Lv SW, Xie M, Feng YQ, Huang WH, Yuan BF. Determination of DNA and RNA methylation in circulating tumor cells by mass spectrometry. Anal Chem. 2016;88:1378–84.

147. Stockert JA, Gupta A, Herzog B, Yadav SS, Tewari AK, Yadav KK. Predictive value of pseudouridine in prostate cancer. Am J Clin Exp Urol. 2019;7:262–72.

148. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. Nature. 1999;402:551–5.

149. Penzo M, Casoli L, Ceccarelli C, Trere D, Ludovini V, Crino L, Montanaro L. DKC1 gene mutations in human sporadic cancer. Histol Histopathol. 2013; 28:365–72.

150. Valleron W, Laprevotte E, Gautier EF, Quelen C, Demur C, Delabesse E, Agirre X, Prosper F, Kiss T, Brousset P. Specific small nucleolar RNA expression profiles in acute leukemia. Leukemia. 2012;26:2052–60.

151. Valleron W, Ysebaert L, Berquet L, Fataccioli V, Quelen C, Martin A, Parrens M, Lamant L, de Leval L, Gisselbrecht C, et al. Small nucleolar RNA expression profiling identifies potential prognostic markers in peripheral T-cell lymphoma. Blood. 2012;120:3997–4005.

152. Ronchetti D, Todoerti K, Tuana G, Agnelli L, Mosca L, Lionetti M, Fabris S, Colapietro P, Miozzo M, Ferrarini M, et al. The expression pattern of small nucleolar and small Cajal body-specific RNAs characterizes distinct molecular subtypes of multiple myeloma. Blood Cancer J. 2012;2:e96.

153. Qin YR, Qiao JJ, Chan TH, Zhu YH, Li FF, Liu H, Fei J, Li Y, Guan XY, Chen L. Adenosine-to-inosine RNA editing mediated by ADARs in esophageal squamous cell carcinoma. Cancer Res. 2014;74:840–51.

154. Chen L, Li Y, Lin CH, Chan TH, Chow RK, Song Y, Liu M, Yuan YF, Fu L, Kong KL, et al. Recoding RNA editing of AZIN1 predisposes to hepatocellular carcinoma. Nat Med. 2013;19:209–16.

155. Yao J, Duan L, Fan M, Yuan J, Wu X. Overexpression of BLCAP induces S phase arrest and apoptosis independent of p53 and NF-kappaB in human tongue carcinoma : BLCAP overexpression induces S phase arrest and apoptosis. Mol Cell Biochem. 2007;297:81–92.

156. Fan DG, Zhao F, Ding Y, Wu MM, Fan QY, Shimizu K, Dohjima T, Nozawa S, Wakahara K, Ohno T, et al. BLCAP induces apoptosis in human Ewing's sarcoma cells. Exp Biol Med (Maywood). 2011;236:1030–5.

157. Chen Y, Wang H, Lin W, Shuai P. ADAR1 overexpression is associated with cervical cancer progression and angiogenesis. Diagn Pathol. 2017;12:12.

158. Hu X, Wan S, Ou Y, Zhou B, Zhu J, Yi X, Guan Y, Jia W, Liu X, Wang Q, et al. RNA over-editing of BLCAP contributes to hepatocarcinogenesis identified

by whole-genome and transcriptome sequencing. Cancer Lett. 2015;357: 510–9.

159. Nakano M, Fukami T, Gotoh S, Nakajima M. A-to-I RNA editing up-regulates human dihydrofolate reductase in breast cancer. J Biol Chem. 2017;292: 4873–84.

160. Chan TH, Qamra A, Tan KT, Guo J, Yang H, Qi L, Lin JS, Ng VH, Song Y, Hong H, et al. ADAR-mediated RNA editing predicts progression and prognosis of gastric cancer. Gastroenterology. 2016;151:637–50 e610.

161. Wang X, Xu Z, Ren X, Chen X, Wei J, Lin W, Li Z, Ou C, Gong Z, Yan Y. Function of low ADARB1 expression in lung adenocarcinoma. PLoS One. 2019;14:e0222298.

162. Cao D, Pizzorno G. Uridine phosophorylase: an important enzyme in pyrimidine metabolism and fluoropyrimidine activation. Drugs Today (Barc). 2004;40:431–43.

163. Cao D, Russell RL, Zhang D, Leffert JJ, Pizzorno G. Uridine phosphorylase (-/-) murine embryonic stem cells clarify the key role of this enzyme in the regulation of the pyrimidine salvage pathway and in the activation of fluoropyrimidines. Cancer Res. 2002;62:2313–7.

164. Guan Y, Bhandari A, Zhang X, Wang O. Uridine phosphorylase 1 associates to biological and clinical significance in thyroid carcinoma cell lines. J Cell Mol Med. 2019;23(11):7438–48.

165. Bader JP, Brown NR, Chiang PK, Cantoni GL. 3-Deazaadenosine, an inhibitor of adenosylhomocysteine hydrolase, inhibits reproduction of Rous sarcoma virus and transformation of chick embryo cells. Virology. 1978;89:494–505.

166. Huang Y, Yan J, Li Q, Li J, Gong S, Zhou H, Gan J, Jiang H, Jia GF, Luo C, Yang CG. Meclofenamic acid selectively inhibits FTO demethylation of m6A over ALKBH5. Nucleic Acids Res. 2015;43:373–84.

Publisher's Note