

m⁶a RNA Methylation: The Implications for Health and Disease

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Abstract

The recent resurgence of interest in m⁶A has been spurred by some intriguing findings detailing the effects and dynamics of this epigenetic modification. The m⁶A modification is a highly reactive and fluid modification which can respond rapidly to a broad variety of stimuli, and translate these signals into cellular activity. The little information that has been established on its functional capacity has opened up many new avenues of research and has tremendous implications for several fields of study. Here we outline the breakthroughs which have led to the resurgence of interest in this modification and discuss the effects and potential they represent in terms of control in the immune system, viral replication and infection, as well as the occurrence and progression of cancer.

Introduction

Analysis of nucleic acid modifications and their explicit effects on epigenetic status is a rapidly expanding research arena, one that is growing in stature and importance. Until recently this field had focussed on changes in the chemistry of DNA and the actions of histone proteins and their subsequent modifications. A few key discoveries are set to add a whole new RNA dimension to this exciting field of research, RNA methylation has entered the fray, specifically N-methyl-6-Adenosine – also known as m⁶A or N6-methyladenosine. Although this is not the only RNA modification, over 100 have been identified; it is by far the most abundant [1]. Not only is this modification found extensively in mRNAs it is also found in non-coding RNAs (ncRNAs), including long ncRNAs (lncRNAs) [2]. The m⁶A modification has been demonstrated to be an internal modification predominantly found in mature mRNA transcripts [2]. Analysis of these transcripts revealed m⁶A modifications on mRNA transcripts which encoded genes involved in transcriptional regulation, RNA metabolism and signalling cascades [2]. The number of 3' UTRs of mRNA transcripts demonstrating m⁶A methylation appear to be above average at two thirds, it should be noted however that accuracy of site detection and methylation state is likely to be more accurate for highly expressed genes than genes with low copy numbers. Around 30% of genes contain microRNA (miRNA) binding sites in the 3' UTRs [3]. Interestingly, where these occur simultaneously the m⁶A modification is found closer to the stop codon, whereas the miRNA binding site appears to be at the opposite end [2]. Without further information this proximity may well be circumstantial and irrelevant, however the occurrence of both together is higher than would be expected by chance alone. Even more intriguingly, some miRNAs themselves contain a m⁶A recognition site which may represent a mechanism for their regulation [4]. The m⁶A modification has been demonstrated in a broad range of species including humans [5], mice [5], virii [6,7], yeast [8-10], bacteria [11] and plants [12]. The methylation reaction is catalysed by a conserved mechanism, based around a multi-component enzymatic complex [13] and counterbalanced by a series of demethylases [14]. Although this multi-component complex has not been elucidated as yet, we postulate that it will behave in a similar manner to other complexes in that it will contain a RNA binding protein (specific for the recognition sequence), although multiple proteins are possible due to the variability of the recognition sequence (12 possible permutations) it seems likely that only 1 or 2 binding proteins are responsible for the core of the complex. Larger numbers of co-factors will determine addition/removal of methylation, leading to several possible complex assemblies. It is also likely that other binding proteins may enhance or repress binding to the recognition sequence or overlapping sites, for example FTO binding sites [15], thus determining which sites are methylation or non-methylated. The recognition sequence for the m⁶A methylation site is significantly different from other RNA modifications, this sequence has been identified as RRACH (where R = G or A and H = A, C or U) [7,16-20]. Its occurrence has been estimated at 1 in 2000 bases in humans [2,5]. It has been found to be located proximal to stop codons and is highly enriched in the 3' untranslated region [2]. Like most methylation modifications of nucleotides S-adenosylmethionine (SAM) acts as a methyl group donor for the reaction [21], linking the methylation of RNA to the dynamic equilibrium of this molecule and its associated enzymes. Due to the critical nature of SAM for methylation of both RNA and DNA it is maintained in a highly regulated equilibrium which responds rapidly to cellular factors to maintain idyllic levels. This also suggests that SAM levels are likely to be a driving force for methylation levels via enzyme activity, where availability of SAM drives methylation and low levels of SAM would stimulate demethylation. This is supported by a recently published model suggesting relative methylation levels is determined by

enzymes [22], the same group also suggest that absolute methylation levels are driven by both enzyme levels/activity and transcription regulation. This model is a little simplistic in its overview as SAM is central to a range of methylation processes not just m⁶A; a more likely model will involve more transcriptional and translational control of methylation co-factors for both relative and absolute m⁶A methylation levels.

Renewed interest in RNA epigenetics

Unlike its more mature sibling, DNA methylation, RNA methylation has not been the subject of intense research or discussion [23]; however, that is about to change. Despite its discovery in the 1970s [24], the m⁶A RNA modification has been mostly neglected until recent times, with research focussing on its occurrence [17] and sequence specificity [16,20] rather than its function.

A new surge of interest was sparked by the discovery that the obesity related (fat mass and obesity related (*Fto*)) gene was capable of demethylating RNA [14]. Since then many new studies identifying novel functions of the m⁶A modification and outlining more members of the methylation/demethylation machinery have been conducted. In this regard, a major component of the m⁶A methylation machinery, METTL3 has been identified as have other candidates for the enzyme complex which drives m⁶A RNA methylation, METTL14 [25], KIAA1429 [26] and WTAP [27]. Furthermore, the activity of a second demethylase, ALKBH5 has also been established. This resurgence in interest has also been accelerated by overcoming a major obstacle in the detection of m⁶A [28], the development of novel techniques and adaptations of old ones, reviewed by Chandola *et al.* (2014) [29]. These have allowed a greater parity of the occurrence and fluidity of this epigenetic modification [30]. There is growing evidence that these RNA modifications can respond rapidly to a variety of stimuli, particularly stress [31,32]. Recently, FTO has been further implicated in the complicated control of RNA epigenetics by evidence which suggests it may catalyse further reactions oxidising the N(6)-methyladenosine to form the intermediate product N(6)-hydroxymethyladenosine, which is followed by the formation of the further oxidised product N(6)-formyladenosine [33]. These modifications represent novel short term (half-life ~ 3 hours [33]) epigenetic changes which may have far reaching implications; they may modulate the effects of the m⁶A modification or may aid in its demethylation, similarly to DNA methylation.

Similarly to DNA methylation, it has been shown that m⁶A RNA methylation can influence gene expression [5,34,35], its regulatory role has been emphasised by its occurrence in only a portion of transcripts [17], with some demonstrating no m⁶A modifications at all [36]. This modification has also been suggested to have other key functions, which include RNA splicing [5,14,37], targeting mRNA for degradation [38], regulating RNA stability by modulating binding of RNA binding proteins [5,39,40], translational control [41], meiosis [8] and cellular differentiation [12]. The consequences of experimental retardation of the methyltransferase system are disparate and range from apoptosis in humans [42], developmental arrest in plants [12] and defective gametogenesis in yeast [9] and fruit flies [43]. Looking for condition specific alterations in m⁶A sites and levels using an activated immune response in bone-marrow derived dendritic cells (BMDCs), embryonic and adult mouse brains, fibroblasts undergoing reprogramming (to induced pluripotent stem cells, iPSCs) and differentiating human embryonic stem cells (ESCs) revealed that methylation profiles were fairly consistent, after controlling for increased specific protein expression under the above conditions [26]. This suggests a similar basal role shared throughout the body. Intriguingly, sites for m⁶A modification are significantly enriched on lower expression genes, with constitutively expressed housekeeping genes (translation, mitochondrial, splicing and chromatin regulation) demonstrating a complete absence of m⁶A methylation [26]. However, the system used for these studies may not be suitable for quantification of levels, while the actual sites may be consistent the occupancy may not be. Mouse embryonic stem cell (mESC) *Mettl3* and *Mettl14* knockdown studies led to a similar phenotype: lack of m⁶A RNA methylation and loss of their self-renewal capability, furthermore, these studies implicated human antigen R (HuR) and microRNA in this process [44].

m⁶A RNA methylation and the brain

The epigenetic status of the brain has been shown to be critical for both development and neurological disorders, including neurodegenerative diseases. DNA methylation has also been shown to be critical in brain development and function [45]. Aberrant cytosine methylation on tRNAs leads to neuro-developmental disorders in mice [46]. m⁶A methylation is also found in tRNAs [47], as well as rRNA [48], the relationship between tRNAs and neural development hints at a potential role for m⁶A methylation in neural development as well. A mouse based study demonstrated a high level of enrichment of m⁶A in liver, kidney and brain [2]. This would suggest that the presence of m⁶A methylation is essential for the development and/or functions of these organs. Levels of this modification in the brain are low during embryogenesis and dramatically increase by adulthood [2]; this suggests that m⁶A plays a role in neuronal maturation and normal functioning of the adult brain. Furthermore, the enrichment in brain and concomitant increases in levels associated with ageing suggests a role in neurodegenerative diseases, for example Alzheimer's disease and Parkinson's disease, however no data is available to support this hypothesis to date. A highly intriguing finding is that a greater percentage of target transcripts for the most highly expressed miRNAs found in the brain contain m⁶A modifications [2]. This raises an interesting question: does m⁶A methylation affect miRNA expression, or do miRNAs influence methylation of their own transcripts? A large number of unique highly likely m⁶A sites were identified in both in the embryonic and adult brain, however closer inspection revealed that this was most likely due to increased expression of particular genes that carry the m⁶A modification [26]. Although it appears the number of sites is not increased in the brain, the levels of m⁶A modified mRNA are increased; this suggests a role for m⁶A in neural function and maturity as the genes that were upregulated and carried m⁶A modifications were involved in neural processes.

Fto deficient mice present with reduced postnatal growth, dysfunctional locomotor control, increased energy usage and lower Insulin growth factor (IGF-1) levels [49], all symptoms associated with DA receptor type 2 (D2R) knockouts [50-52]. A recent study has outlined a critical role for *Fto* in the regulation of dopamine (DA) signalling in the midbrain [53]. The link between this demethylase and DA signalling immediately suggests a role for m⁶A methylation as well. Hess *et al.* go further to demonstrate increased m⁶A modifications present in mRNAs encoding key components of neuronal signalling, many from the DA pathway itself. This further translated into disrupted expression of these proteins [53]. This indicates that m⁶A methylation plays a critical role in regulating gene transcription and expression of key components in DA signalling, under the direct control of the FTO protein. The links between this protein and DA further suggest a role in Parkinson's disease, where DA disruption is central to the phenotype of this neurodegenerative disorder. Variants of the *Fto* gene are known to associate with childhood and adult obesity [54-56], however it has also been recently associated with reduced brain volume in healthy elderly individuals [57], attention deficit disorders [58] and possibly addiction [59], summarised in Table 1. Similarly to m⁶A levels the *Fto* gene is upregulated in neurons throughout the brain [60], it also appears that it is regulated by levels of essential amino acids [61]. These facts link nutritional stress with the DA system and m⁶A modification of RNA. It is possible that RNA methylation provides a mechanism for rapid response during nutritional stress. Overexpression of FTO in cultured cells led to a reduction in m⁶A found in mRNA [2,5,14]. However, its overexpression in a mouse model increased the levels of the methylation machinery components but did not appear to alter levels of the m⁶A modification present [62]. It has been demonstrated that FTO control of translation, via demethylation of specific mRNA subsets, is dependent on its enzymatic function [63]. Although FTO is one of the major players in the regulation of m⁶A modification of RNA, it would appear that a secondary signal is required to regulate its activity possibly in response to nutritional stress. With regard to FTO activity in the brain it has recently been linked, along with the m⁶A modification of RNA, to epilepsy [4].

Gene/Protein	Proposed Function in m ⁶ A	Related diseases/disorders
FTO	Demethylation	Parkinson's diseases, Attention Deficit Disorder, addiction, epilepsy, viral infection, colorectal cancer, endometrial cancer, stomach cancer, prostate cancer, breast cancer and pancreatic cancer
ALKBH5	Demethylation	Cancer (other family members implicated in bladder cancer)
WTAP	Methylation	Leukemia, localised inflammation
METTL3	Methylation	Cancer (via DNA damage repair pathways)
METTL14	Methylation	Cancer (via regulation of self-renewal capacity)

Table 1: Summary of known m⁶A methylation enzymes and their relationship with disease/disorders

m⁶A RNA methylation and the immune system

The influence of *Fto* and the m⁶A modification on the DA signalling system gives it a broad reach, not least in the function and activity of the immune system. For instance the D2-like receptors, which are particularly influenced by m⁶A modifications [53], are found in greater levels on memory and effector CD4⁺ T lymphocytes than their naive counterparts [64]. Furthermore, this influence extends to the development of T cells, particularly in the thymus [65]. The D2-like DA receptors are also capable of attenuating NK cell activity [66]. These receptors are considered to be more important for the modulation of T lymphocyte function than D1-like DA receptors [67], this indicates a greater degree of control over T lymphocytes may be exerted by the m⁶A modification and its machinery than had previously been thought possible. Dopamine has also been shown to upregulate interleukin (IL)-6 and IL-8 in keratinocytes [68], indicating a broader control over inflammation. D2-like receptors have been demonstrated to exhibit an influence over chronic neuroinflammation [69] which implicates the m⁶A modification in neurodegeneration and its associated disorders. WTAP influences cellular immune responses in patients with leukemia [70], administration of a vaccine based on WTAP also results in localised skin inflammation around the injection sites [71], providing further evidence for a role of m⁶A modification in the modulation of inflammation.

Interestingly, BMDCs stimulated with lipopolysaccharide (LPS) demonstrated the presence of a large number of highly likely m⁶A sites these related to increased expression of proteins involved in the immune response [26]. This indicates a key role for this modification in the direct modulation of immune response elements and thus the immune system, particularly an active response. It has also been demonstrated that LPS challenge down-regulates FTO mRNA levels, at least in the liver this is in conjunction with a reduction of Toll-like receptor (TLR) 2 and 4 levels [72].

The TLRs recognise a variety of antigens from infectious agents to damaged cells from the host organism and initiate a response [73]. Three of these recognise RNA: TLR3 recognises double stranded RNA (dsRNA) [74,75], whereas TLR7 and TLR8 recognise single stranded RNA (ssRNA) [76,77] or double stranded short interfering RNAs (siRNAs) [78]. It has now been shown that methylated RNAs, including the m⁶A modification, are significantly less immunogenic [79]. This may be part of the self-recognition machinery to prevent TLRs from activating upon binding of native mRNAs.

Viral use of m⁶A RNA methylation

The lack of immune response to methylated RNA via the TLR pathways provides an ideal sanctuary for RNA based virii. The use of the m⁶A modification on their genome would prevent detection by the TLRs which are part of the frontline defence against pathogens [80]. It is therefore unsurprising to find some RNA based viruses which utilise this modification, for example Rous Sarcoma Virus (RSV) contains 7 m⁶A methylation sites in its genome [6] and simian virus 40 (SV40) has more than 10 sites [7]. This modification has also been identified in B77 avian sarcoma virus [81] and Adenovirus type-2 [82]. It would also appear that m⁶A RNA methylation is more densely utilised in these viral genomes than the mammalian genome with frequencies ranging from 1 in 400 found in adenovirus type-2 [82] to 1 in 1000 found in RSV [83,84]. The m⁶A modifications in SV40 were not only found in the SV40 nuclear RNA, but also specifically on the 16S and 19S viral messengers [7], this may be a modification to protect or localise these mRNAs and the nuclear RNA in host cells. Data from Adenovirus type-2 demonstrates conservation of the m⁶A modification between the nuclear resident viral genome and the mRNA transcripts it generates [85]. Furthermore, as mentioned previously these modifications may be an evolutionary attempt to evade the host immune system by avoiding detection by TLRs. Given the critical nature of the m⁶A methylation to some virii, it is likely that these directly or indirectly influence host m⁶A methylation/demethylation to further their own ends; the restricted size of the viral genomes would suggest that this is accomplished via viral co-factors or viral driven expression of host co-factors.

Interestingly, there are several RNA based viruses which produce mRNAs that have been shown not to possess any m⁶A modifications; these include tobacco mosaic virus [83], reovirus [84], vaccinia virus [86], cytoplasmic polyhedrosis virus [87] and Newcastle disease virus [88]. Investigations into the occurrence and influence of this modification in viral genomes peaked in the 1970s, given the latest reports of the occurrence and functionality of m⁶A RNA methylation perhaps a new wave of research will investigate the incorporation of this epigenetic mark in virii – is it a protective measure against internal degradation, a mechanism to avoid the host immune system or an enhancer to ensure translation of viral mRNAs to enhance the lytic phase.

Cancer and m⁶A RNA methylation

Given that m⁶A modifications are found on many housekeeping genes which influence translation, energy production/usage and differentiation and that it can stabilise mRNAs it is highly likely that this modification will be involved in cancer at some juncture; whether this is a key step in the formation, advancement or malignancy of tumours has yet to be seen. It is however, evident that this modification will become the subject of significant levels of research and spark some heated debates in the near future. Perhaps it will lead to new understanding, diagnosis and treatments for a variety of cancer subtypes.

Evidence for the involvement of m⁶A mRNA modifications in cancer is already beginning to accumulate, although most is circumstantial at this point. For instance, widely disparate levels are evident in a variety of cancer cell lines [2], this indicates that this normally fairly consistent modification is highly disrupted, at least in some cancers, whether this is cause or effect remains to be seen. Additionally, increasing levels of SAM can inhibit the growth of some human gastric cancer cells; this effect is mediated through a reduction in mRNA levels, and thus protein levels, of c-myc and urokinase plasminogen activator (uPA) [89]. Although levels of SAM will affect many forms of methylation, its effects on mRNA expression may indicate a role for m⁶A in targeting specific mRNAs for degradation thus lowering their levels; it would require further experimentation to fully elucidate the effects of SAM on these cells. Intriguingly, it has been shown that inhibition of the m⁶A modification leads to a prolonged circadian cycle [90] and that disruption of the circadian clock may be associated with the development of cancer in some cases [91].

Evidence for the involvement of the m⁶A RNA methylation associated machinery is more prolific, WTAP is a key antigen in leukemia [70]. FTO is mutated in ~82% of colorectal cancer cases, 100% of colorectal carcinoma cell cultures, 41.7% of endometrial carcinomas and 6.7% of stomach cancer cases [92]. Allelic variants of FTO have also been associated with prostate cancer risk [93], breast cancer [94,95] and pancreatic cancer [96]. The demonstration that METTL3 is phosphorylated when DNA damage is detected [97] indicates it may play a role in the cellular reaction to DNA damage. Although no direct evidence has been presented, the role of these pathways in many tumour types suggests that METTL3 may play a role in tumourigenesis. Similarly, no direct evidence exists for a role of ALKBH5 in tumourigenesis, however other members of its family, namely ALKBH2 and ALKBH8, have been implicated in bladder cancer progression [98,99].

Viral driven tumourigenesis is not uncommon, for example human papillomavirus [100], with the potential for RNA viruses to evade the immune system using the m⁶A modification there is the opportunity for this process to occur unabated. The activation of TLR signalling also induces several anti-cancer proteins and is considered a target for cancer therapy [101]. Furthermore, modification of RNA bases has been shown to enhance/perturb TLR signalling pathways [100,102], leading to the possibility that RNA modification plays a role in cancer progression [29].

The influence of other epigenetic modifications in tumourigenesis is unquestionable; in fact DNA methylation disruption is considered one of the hallmarks of cancer [103]. These changes are so prolific in tumourigenesis that DNA epigenetic modifications, DNA methylation and histone acetylation/deacetylation, are already being targeted for use in cancer therapy [104], perhaps RNA epigenetics is the next avenue for investigation in the pursuit of novel therapeutics.

References

1. Cantara WA, Crain PF, Rozenski J, McCloskey JA, Harris KA, et al. (2011) The RNA Modification Database, RNAMDB: 2011 update. *Nucleic Acids Res* 39: 195-201.
2. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, et al. (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* 149: 1635-46.
3. Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120: 15-20.
4. Rowles J, Wong M, Powers R, Olsen M (2012) FTO, RNA epigenetics and epilepsy. *Epigenetics* 7: 1094-7.
5. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, et al. (2012) Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature* 485: 201-6.
6. Kane SE, Beemon K (1985) Precise localization of m6A in Rous sarcoma virus RNA reveals clustering of methylation sites: implications for RNA processing. *Mol Cell Biol* 5: 2298-306.
7. Canaani D, Kahana C, Lavi S, Groner Y (1979) Identification and mapping of N6-methyladenosine containing sequences in simian virus 40 RNA. *Nucleic Acids Res* 6: 2879-99.
8. Schwartz S, Agarwala SD, Mumbach MR, Jovanovic M, Mertins P, et al. (2013) High-resolution mapping reveals a conserved, widespread, dynamic mRNA methylation program in yeast meiosis. *Cell* 155: 1409-21.
9. Clancy MJ, Shambaugh ME, Timpte CS, Bokar JA (2002) Induction of sporulation in *Saccharomyces cerevisiae* leads to the formation of N6-methyladenosine in mRNA: a potential mechanism for the activity of the IME4 gene. *Nucleic Acids Res* 30: 4509-18.
10. Bodi Z, Button JD, Grierson D, Fray RG (2010) Yeast targets for mRNA methylation. *Nucleic Acids Res* 38: 5327-35.
11. Sergiev PV, Serebryakova MV, Bogdanov AA, Dontsova OA (2008) The ybiN gene of *Escherichia coli* encodes adenine-N6 methyltransferase specific for modification of A1618 of 23 S ribosomal RNA, a methylated residue located close to the ribosomal exit tunnel. *J Mol Biol* 375: 291-300.
12. Zhong S, Li H, Bodi Z, Button J, Vespa L, et al. (2008) MTA is an Arabidopsis messenger RNA adenosine methylase and interacts with a homolog of a sex-specific splicing factor. *Plant Cell* 20: 1278-88.
13. Agarwala SD, Blitzblau HG, Hochwagen A, Fink GR (2012) RNA methylation by the MIS complex regulates a cell fate decision in yeast. *PLoS Genet* 8: e1002732.
14. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, et al. (2011) N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat Chem Biol* 7: 885-7.
15. Liu L, Zhang SW, Zhang YC, Liu H, Zhang L, et al. (2014) Decomposition of RNA methylome reveals co-methylation patterns induced by latent enzymatic regulators of the epitranscriptome. *Mol Biosyst* 1: 262-74.
16. Harper JE, Miceli SM, Roberts RJ, Manley JL (1990) Sequence specificity of the human mRNA N6-adenosine methylase in vitro. *Nucleic Acids Res* 18: 5735-41.
17. Horowitz S, Horowitz A, Nilsen TW, Munns TW, Rottman FM (1984) Mapping of N6-methyladenosine residues in bovine prolactin mRNA. *Proc Natl Acad Sci USA* 81: 5667-71.
18. Csepány T, Lin A, Baldick CJ, Beemon K (1990) Sequence specificity of mRNA N6-adenosine methyltransferase. *J Biol Chem* 265: 20117-22.
19. Rottman FM, Bokar JA, Narayan P, Shambaugh ME, Ludwiczak R (1994) N6-adenosine methylation in mRNA: substrate specificity and enzyme complexity. *Biochimie* 76: 1109-14.
20. Wei CM, Moss B (1977) Nucleotide sequences at the N6-methyladenosine sites of HeLa cell messenger ribonucleic acid. *Biochemistry* 16: 1672-6.
21. Wertheimer AM, Chen SY, Borchardt RT, Furuichi Y (1980) S-Adenosylmethionine and its analogs. Structural features correlated with synthesis and methylation of mRNAs of cytoplasmic polyhedrosis virus. *J Biol Chem* 255: 5924-30.
22. Meng J, Lu Z, Liu H, Zhang L, Zhang S, et al. (2014) A protocol for RNA methylation differential analysis with MeRIP-Seq data and exomePeak R/Bioconductor package. *Methods* 69: 274-81.
23. He C (2010) Grand challenge commentary: RNA epigenetics? *Nat Chem Biol* 6: 863-5.
24. Desrosiers R, Friderici K, Rottman F (1974) Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. *Proc Natl Acad Sci U S A* 71: 3971-5.
25. Liu J, Yue Y, Han D, Wang X, Fu Y, et al. (2013) A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. *Nat Chem Biol* 10: 93-5.
26. Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, et al. (2014) Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. *Cell Rep* 8: 284-96.
27. Ping XL, Sun BF, Wang L, Xiao W, Yang X, et al. (2014) Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. *Cell Res* 24: 177-89.
28. Dai Q, Fong R, Saikia M, Stephenson D, Yu YT, et al. (2007) Identification of recognition residues for ligation-based detection and quantitation of pseudouridine and N6-methyladenosine. *Nucleic Acids Res* 35: 6322-9.
29. Chandola U, Das R, Panda B (2014) Role of the N6-methyladenosine RNA mark in gene regulation and its implications on development and disease. *Brief Funct Genomics*.
30. Jia G, Fu Y, He C (2013) Reversible RNA adenosine methylation in biological regulation. *Trends Genet* 29: 108-15.
31. Chan CT, Dyavaiah M, DeMott MS, Taghizadeh K, Dedon PC, et al. (2010) A quantitative systems approach reveals dynamic control of tRNA modifications during cellular stress. *PLoS Genet* 6: e1001247.
32. Schaefer M, Pollex T, Hanna K, Tuorto F, Meusburger M, et al. (2010) RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. *Genes Dev* 24: 1590-5.
33. Fu Y, Jia G, Pang X, Wang RN, Wang X, et al. (2013) FTO-mediated formation of N6-hydroxymethyladenosine and N6-formyladenosine in mammalian RNA. *Nat Commun* 4: 1798.
34. Zheng G, Dahl JA, Niu Y, Fu Y, Klungland A, et al. (2013) Sprouts of RNA epigenetics: the discovery of mammalian RNA demethylases. *RNA Biol* 10: 915-8.
35. Fu Y, Dominissini D, Rechavi G, He C (2014) Gene expression regulation mediated through reversible m⁶A RNA methylation. *Nat Rev Genet* 15: 293-306.
36. Perry RP, Scherrer K (1975) The methylated constituents of globin mRNA. *FEBS Lett* 57: 73-8.
37. Bokar JA, Shambaugh ME, Polayes D, Matera AG, Rottman FM (1997) Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. *RNA* 3: 1233-47.

38. Harigaya Y, Tanaka H, Yamanaka S, Tanaka K, Watanabe Y, et al. (2006) Selective elimination of messenger RNA prevents an incidence of untimely meiosis. *Nature* 442: 45-50.
39. Zhang Z, Theler D, Kaminska KH, Hiller M, de la Grange P, et al. (2010) The YTH domain is a novel RNA binding domain. *J Biol Chem* 285: 14701-10.
40. Brennan CM, Steitz JA (2001) HuR and mRNA stability. *Cell Mol Life Sci* 58: 266-77.
41. Tuck MT, Wiehl PE, Pan T (1999) Inhibition of 6-methyladenine formation decreases the translation efficiency of dihydrofolate reductase transcripts. *Int J Biochem Cell Biol* 31: 837-51.
42. Bokar JA (2005) Fine Tuning of RNA Functions by Modification and Editing. ed. Grosjean H Springer 12: 141-177.
43. Hongay CF, Orr-Weaver TL (2011) Drosophila Inducer of MEiosis 4 (IME4) is required for Notch signaling during oogenesis. *Proc Natl Acad Sci U S A* 108: 14855-60.
44. Wang Y, Li Y, Toth JJ, Petroski MD, Zhang Z, et al. (2014) N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. *Nat Cell Biol* 16: 191-8.
45. Kobow K, Blümcke I (2014) Epigenetic mechanisms in epilepsy. *Prog Brain Res* 213: 279-316.
46. Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, et al. (2014) Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. *EMBO J* 33: 2020-39.
47. Saneyoshi M, Harada F, Nishimura S (1969) Isolation and characterization of N6-methyladenosine from Escherichia coli valine transfer RNA. *Biochim Biophys Acta* 190: 264-73.
48. Iwanami Y, Brown GM (1968) Methylated bases of ribosomal ribonucleic acid from HeLa cells. *Arch Biochem Biophys* 126: 8-15.
49. Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, et al. (2009) Inactivation of the Fto gene protects from obesity. *Nature* 458: 894-8.
50. Diaz-Torga G, Feierstein C, Libertun C, Gelman D, Kelly MA, et al. (2002) Disruption of the D2 dopamine receptor alters GH and IGF-I secretion and causes dwarfism in male mice. *Endocrinology* 143: 1270-9.
51. Sibley DR (1999) New insights into dopaminergic receptor function using antisense and genetically altered animals. *Annu Rev Pharmacol Toxicol* 39: 313-41.
52. Beaulieu JM, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 63: 182-217.
53. Hess ME, Hess S, Meyer KD, Verhagen LA, Koch L, et al. (2013) The fat mass and obesity associated gene (Fto) regulates activity of the dopaminergic midbrain circuitry. *Nat Neurosci* 16: 1042-8.
54. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, et al. (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316: 889-94.
55. Yeo GS (2012) FTO and obesity: a problem for a billion people. *J Neuroendocrinol* 24: 393-4.
56. Dina C, Meyre D, Gallina S, Durand E, Körner A, et al. (2007) Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* 39: 724-6.
57. Ho AJ, Stein JL, Hua X, Lee S, Hibar DP, et al. (2010) A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. *Proc Natl Acad Sci U S A* 107: 8404-9.
58. Choudhry Z, Sengupta SM, Grizenko N, Thakur GA, Fortier ME, et al. (2013) Association between obesity-related gene FTO and ADHD. *Obesity (Silver Spring)* 21: E738-44.
59. Sobczyk-Kopciol A, Broda G, Wojnar M, Kurjata P, Jakubczyk A, et al. (2010) Inverse association of the obesity predisposing FTO rs9939609 genotype with alcohol consumption and risk for alcohol dependence. *Addiction* 106: 739-48.
60. McTaggart JS, Lee S, Iberl M, Church C, Cox RD, et al. (2011) FTO is expressed in neurones throughout the brain and its expression is unaltered by fasting. *PLoS One* 6: e27968.
61. Cheung MK, Gulati P, O'Rahilly S, Yeo GS (2013) FTO expression is regulated by availability of essential amino acids. *Int J Obes (Lond)* 37: 744-7.
62. Merksteiner M, McTaggart JS, Lee S, Kramer HB, McMurray F, et al. (2014) Changes in gene expression associated with FTO overexpression in mice. *PLoS One* 9: e97162.
63. Gulati P, Cheung MK, Antrobus R, Church CD, Harding HP, et al. (2013) Role for the obesity-related FTO gene in the cellular sensing of amino acids. *Proc Natl Acad Sci USA* 110: 2557-62.
64. Kustrimovic N, Rasini E, Legnaro M, Marino F, Cosentino M (2014) Expression of dopaminergic receptors on human CD4+ T lymphocytes: flow cytometric analysis of naive and memory subsets and relevance for the neuroimmunology of neurodegenerative disease. *J Neuroimmune Pharmacol* 9: 302-12.
65. Mignini F, Sabbatini M, Capacchietti M, Amantini C, Bianchi E, et al. (2013) T-cell subpopulations express a different pattern of dopaminergic markers in intra- and extra-thymic compartments. *J Biol Regul Homeost Agents* 27: 463-75.
66. Zhao W, Huang Y, Liu Z, Cao BB, Peng YP, et al. (2013) Dopamine receptors modulate cytotoxicity of natural killer cells via cAMP-PKA-CREB signaling pathway. *PLoS One* 8: e65860.
67. Huang Y, Qiu AW, Peng YP, Liu Y, Huang HW, et al. (2010) Roles of dopamine receptor subtypes in mediating modulation of T lymphocyte function. *Neuro Endocrinol Lett* 31: 782-91.
68. Parrado AC, Canellada A, Gentile T, Rey-Roldán EB. (2012) Dopamine agonists upregulate IL-6 and IL-8 production in human keratinocytes. *Neuroimmunomodulation* 19: 359-66.
69. Shao W, Zhang SZ, Tang M, Zhang XH, Zhou Z, et al. (2012) Suppression of neuroinflammation by astrocytic dopamine D2 receptors via α B-crystallin. *Nature* 494: 90-4.
70. Casalegno-Garduño R, Schmitt A, Wang X, Xu X, Schmitt M (2010) Wilms' tumor 1 as a novel target for immunotherapy of leukemia. *Transplant Proc* 42: 3309-11.
71. Soeda A, Morita-Hoshi Y, Kaida M, Wakeda T, Yamaki Y, et al. (2010) Long-term administration of Wilms tumor-1 peptide vaccine in combination with gemcitabine causes severe local skin inflammation at injection sites. *Jpn J Clin Oncol* 40: 1184-8.
72. Zhang Y, Guo F, Ni Y, Zhao R (2013) LPS-induced inflammation in the chicken is associated with CCAAT/enhancer binding protein beta-mediated fat mass and obesity associated gene down-regulation in the liver but not hypothalamus. *BMC Vet Res* 9: 257.
73. Akira S, Takeda K (2004) Toll-like receptor signalling. *Nat Rev Immunol* 4: 499-511.
74. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413: 732-8.
75. Karikó K, Ni H, Capodici J, Lamphier M, Weissman D (2004) mRNA is an endogenous ligand for Toll-like receptor 3. *J Biol Chem* 279: 12542-50.

76. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, et al. (2004) Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 303: 1526-9.
77. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 303: 1529-31.
78. Hornung V, Guenther-Biller M, Bourquin C, Ablasser A, Schlee M, et al. (2005) Sequence-specific potent induction of IFN- α by short interfering RNA in plasmacytoid dendritic cells through TLR7. *Nat Med* 11: 263-70.
79. Karikó K, Buckstein M, Ni H, Weissman D (2005) Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* 23: 165-75.
80. Medzhitov R (2001) Toll-like receptors and innate immunity. *Nat Rev Immunol* 1: 135-45.
81. Dimock K, Stoltzfus CM (1977) Sequence specificity of internal methylation in B77 avian sarcoma virus RNA subunits. *Biochemistry* 16: 471-8.
82. Sommer S, Salditt-Georgieff M, Bachenheimer S, Darnell JE, Furuichi Y, et al. (1976) The methylation of adenovirus-specific nuclear and cytoplasmic RNA. *Nucleic Acids Res* 3: 749-65.
83. Beemon K, Keith J (1977) Localization of N6-methyladenosine in the Rous sarcoma virus genome. *J Mol Biol* 113: 165-79.
84. Furuichi Y, Shatkin AJ, Stavnezer E, Bishop JM (1975) Blocked, methylated 5'-terminal sequence in avian sarcoma virus RNA. *Nature* 257: 618-20.
85. Chen-Kiang S, Nevins JR, Darnell JE (1979) N-6-methyl-adenosine in adenovirus type 2 nuclear RNA is conserved in the formation of messenger RNA. *J Mol Biol* 135: 733-52.
86. Wei CM, Moss B (1975) Methylated nucleotides block 5'-terminus of vaccinia virus messenger RNA. *Proc Natl Acad Sci U S A* 72: 318-22.
87. Furuichi Y, Miura K (1975) A blocked structure at the 5' terminus of mRNA from cytoplasmic polyhedrosis virus. *Nature* 253: 374-5.
88. Colonno RJ, Stone HO (1975) Methylation of messenger RNA of Newcastle disease virus in vitro by a virion-associated enzyme. *Proc Natl Acad Sci USA* 72: 2611-5.
89. Zhao Y, Li JS, Guo MZ, Feng BS, Zhang JP (2010) Inhibitory effect of S-adenosylmethionine on the growth of human gastric cancer cells in vivo and in vitro. *Chin J Cancer* 29: 752-60.
90. Fustin JM, Doi M, Yamaguchi Y, Hida H, Nishimura S, et al. (2013) RNA-methylation-dependent RNA processing controls the speed of the circadian clock. *Cell* 155: 793-806.
91. Zelinski EL, Deibel SH, McDonald RJ (2014) The trouble with circadian clock dysfunction: multiple deleterious effects on the brain and body. *Neurosci Biobehav Rev* 40: 80-101.
92. Linnebacher M, Wienck A, Boeck I, Klar E (2010) Identification of an MSI-H tumor-specific cytotoxic T cell epitope generated by the (-1) frame of U79260(FTO). *J Biomed Biotechnol* 2010: 841451.
93. Machiela MJ, Lindström S, Allen NE, Haiman CA, Albanes D, et al. (2012) Association of type 2 diabetes susceptibility variants with advanced prostate cancer risk in the Breast and Prostate Cancer Cohort Consortium. *Am J Epidemiol* 176: 1121-9.
94. Long J, Zhang B, Signorello LB, Cai Q, Deming-Halverson S, et al. (2013) Evaluating genome-wide association study-identified breast cancer risk variants in African-American women. *PLoS One* 8(4): e58350.
95. Kaklamani V, Yi N, Sadim M, Siziopikou K, Zhang K, et al. (2011) The role of the fat mass and obesity associated gene (FTO) in breast cancer risk. *BMC Med Genet* 12: 52.
96. Pierce BL, Austin MA, Ahsan H (2011) Association study of type 2 diabetes genetic susceptibility variants and risk of pancreatic cancer: an analysis of PanScan-I data. *Cancer Causes Control* 22: 877-83.
97. Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER, Hurov KE, et al. (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316: 1160-6.
98. Shimada K, Nakamura M, Anai S, De Velasco M, Tanaka M, et al. (2009) A novel human AlkB homologue, ALKBH8, contributes to human bladder cancer progression. *Cancer Res* 69: 3157-64.
99. Fujii T, Shimada K, Anai S, Fujimoto K, Konishi N (2013) ALKBH2, a novel AlkB homologue, contributes to human bladder cancer progression by regulating MUC1 expression. *Cancer Sci* 104: 321-7.
100. Zhou Q, Zhu K, Cheng H (2013) Toll-like receptors in human papillomavirus infection. *Arch Immunol Ther Exp* 61: 203-15.
101. Wolska A, Lech-Marañda E, Robak T (2009) Toll-like receptors and their role in carcinogenesis and anti-tumor treatment. *Cell Mol Biol Lett* 14: 248-72.
102. Shchelyakov DV, Logunov DY, Tukhvatulin AI, Shmarov MM, Naroditsky BS, et al. (2010) Toll-Like Receptors (TLRs): The Role in Tumor Progression. *Acta Naturae* 2: 21-9.
103. Jones PA, Baylin SB (2007) The epigenomics of cancer. *Cell* 128: 683-92.
104. Ferguson LR, Tatham AL, Lin Z, Denny WA (2011) Epigenetic regulation of gene expression as an anticancer drug target. *Curr Cancer Drug Targets* 11: 199-212.

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