Journal Pre-proof

Environmental Epitranscriptomics

Akin Cayir, Hyang-Min Byun, Timothy M. Barrow

PII: S0013-9351(20)30780-5

DOI: https://doi.org/10.1016/j.envres.2020.109885

Reference: YENRS 109885

To appear in: *Environmental Research*

Received Date: 30 March 2020

Revised Date: 25 June 2020

Accepted Date: 28 June 2020

Please cite this article as: Cayir, A., Byun, H.-M., Barrow, T.M., Environmental Epitranscriptomics, *Environmental Research*, https://doi.org/10.1016/j.envres.2020.109885.

Environmental Epitranscriptomics

Akin Cayir[1*], Hyang-Min Byun[2], Timothy M. Barrow[3]

[1] Vocational Health College, Canakkale Onsekiz Mart University, Canakkale, Turkey

[2] Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

[3] Faculty of Health Sciences and Wellbeing, University of Sunderland, Sunderland, United Kingdom

* Corresponding author: Akin Cayir

Vocational Health College, Canakkale Onsekiz Mart University, Canakkale, Turkey e-mail: acincay79@yahoo.com

Tel: +90 505 477 04 23

Fax: +90 286 212 88 00

Abstract

Chemical modifications of RNA molecules have gained increasing attention since evidence emerged for their substantive roles in a range of biological processes, such as the stability and translation of mRNA transcripts. More than 150 modifications have been identified in different organisms to date, collectively known as the 'epitranscriptome', with 6-methyladenosine (m^6A), 5-methylcytidine (m^5C), pseudouridine and N1-methyladenosine (m^1A) the most extensively investigated. Although we are just beginning to elucidate the roles of these modifications in cellular functions, there is already evidence for their dysregulation in diseases such as cancer and neurodevelopmental disorders. There is currently more limited knowledge regarding how environmental exposures affect the epitranscriptome and how this may mediate disease risk, but evidence is beginning to emerge. Here, we review the current evidence for the impact of environmental exposures such as benzo[a]pyrene, bisphenol A, pesticides, metals and nanoparticles upon RNA modifications and the expression of their 'writers' (methyl transferases), 'erasers' (demethylases) and 'readers' . We discuss future directions of the field and identify areas of particular promise and consider the technical challenges that are faced.

Keywords: RNA modifications; epitranscriptomics; environmental exposure; m^6A; m^1A; m^5C

1. Introduction

Environmental epigenetics studies the effects of environmental exposures upon the epigenome in relation to human health and disease risk. There is now extensive evidence to suggest that a variety of environmental exposures alter epigenetic marks such as DNA 5-methylcytocine (m^5C) and histone modifications, thereby potentially modulating gene transcription. It is now recognised that RNA molecules undergo similar modifications. To date, more than 150 different modifications of coding and non-coding RNAs have been reported (Boccaletto et al., 2018), collectively referred to as "*RNA epigenetics*" (He, 2010) or the "*epitranscriptome*" (Saletore et al., 2012). Similar to DNA and histone modifications, RNA modifications are maintained by proteins serving as writers, readers, and erasers. "Writers" are comprised of transferase enzymes that facilitate addition of chemical groups to RNA bases, such as METTL3 (methyltransferase like 3), METTL14 (methyltransferase like 14), METTL16 (methyltransferase like 16), RBM15 (RNA binding motif protein 15), WTAP (wilms tumor 1-associating protein), and KIAA1429(vir like m^6A methyltransferase associated). These are removed by "erasers", such as FTO/ALKBH9 (alpha-ketoglutarate dependent dioxygenase) and ALKBH5 (alkb homolog 5, RNA demethylase). The RNA modifications are recognised and utilised by "readers", such as HNRNPC (heterogeneous nuclear ribonucleoprotein C), HNRNPA2B1 (heterogeneous nuclear ribonucleoprotein A2/B1), YTHDF2 (YTH N6-Methyladenosine RNA binding protein 2), YTHDF1 (YTH N6-Methyladenosine RNA binding protein 1), and eIF3 (eukaryotic initiation factor 3). The most widely studied of these to date are those which modulate m^6A, m^5C, and m^1A RNA methylation.

Epitranscriptomics is emerging as a field of great interest. As with epigenetics, much of the early research has focused on human diseases, especially cancer. However, there is

currently much more limited data available regarding how environmental exposures alter RNA modifications. Here, we review the evidence for the alteration of RNA modifications in disease and in response to a range of exposures, evaluating the work from *in vitro*, animal model and human population studies.

2. Types of RNA modification

The first modification in RNAs was discovered in 1957 (Davis and Allen, 1957). Since then, more than 150 different RNA modifications have been reported in a wide range of organisms (Gilbert et al., 2016), including m^6A, N6,2'-O-dimethyladenosine, m^5C, 5-hydroxylmethylcytidine (hm^5C), inosine, pseudouridine, and m^1A in eukaryotic organisms (Dominissini et al., 2012; Meyer et al., 2012). Here, we will briefly introduce three of the most widely studied modifications m^6A, m^1A and m^5C (*Figure 1*), that through transcriptome-wide studies have been demonstrated to be present in tRNAs, rRNAs, mRNAs, long noncoding RNAs, miRNAs, and circRNAs (Dominissini et al., 2012; Meyer et al., 2012).

2.1 6-methyladenosine (m^6A)

m^6A was firstly discovered in polyadenylated RNA in mammalian cells in 1970, and it was the first RNA modification to have its writers, erasers and readers characterised (Cao et al., 2016). It is highly abundant in mRNA, more so than any other mRNA modification, with two recent studies identifying m^6A modifications in the mRNAs of over 7,600 protein-coding genes and more than 300 non-coding RNAs, with significant enrichment within the 3' UTR (Dominissini et al., 2012; Meyer et al., 2012). This modification is associated with RNA splicing (Little et al., 2000; Ping et al., 2014; Zheng et al., 2013), stability (Geula et al., 2015; Wang et al., 2014) and mRNA decay (Cao et al., 2016), and therefore is important in post-

transcriptional regulation of gene expression. The writers and readers of m^6A modifications have been linked to specific biological functions, with WTAP involved in mRNA splicing (Little et al., 2000; Ping et al., 2014; Zheng et al., 2013), YTHDF1 and eIF3 in the translation process (Meyer et al., 2015; Wang et al., 2015), and YTHDF2 in mRNA decay (Cao et al., 2016). Subsequently, disruptions of those genes that control RNA m^6A methylation levels are related to human diseases and disorders such as obesity, type 2 diabetes (Dina et al., 2007; Frayling et al., 2007; Klungland and Dahl, 2014; Scuteri et al., 2007) and neurological diseases (McGuinness and McGuinness, 2014), while changes in m^6A levels have been identified in infertility, carcinogenesis, meiosis, and in relation to stemness (Fu et al., 2014).

2.2 N1-methyladenosine (m^1A)

First reported in 1961 (Dunn, 1961), the m^1A RNA modification has been identified in tRNA (RajBhandary et al., 1966), rRNA (Sharma et al., 2013), mRNA (Dominissini et al., 2016) and in mitochondrial (mt) transcripts (Li et al., 2017a), yet its various biological functions have only recently begun to be established. In humans, the m^1A modification at position 58 of tRNA molecules is written by the TRMT6/TRMT61 heterodimer and demethylated by ALKBH1, and it serves to promote the initiation of translation (Liu et al., 2016). m^1A in mRNA is also believed to impact upon translation, with modifications near to the 5′ cap and 5′ UTR in nuclear transcripts promoting their translation (Li et al., 2017a), but elsewhere it may inhibit translation by interruption of Watson-Crick base pairing (Li et al., 2017a; Morena et al., 2018). Nuclear and mitochondrial RNA molecules are chemically modified by distinct enzymes, with nuclear m^1A in pre-tRNAs and some pre-mRNAs written by the tRNA m^1A methyltransferase complex TRMT6/TRMT61A and erased by AlkB homolog proteins ALKBH1 and ALKBH3, while in mitochondria mt-tRNAs and a subset of mt-mRNAs are methylated by the mt-tRNA m^1A methyltransferases TRMT61B and TRMT10C.

2.3 5-methylcytocine (m^5C)

The m^5C modification is one of the most common in RNAs. Recent high-throughput RNA methylation profiling by bisulfite sequencing in HeLa cells has identified m^5C at more than 240 sites in tRNAs and 10,275 candidate sites in mRNAs and non-coding RNAs (Jühling et al., 2009; Squires et al., 2012). In contrast, there appear to be relatively fewer m^5C residues in eukaryotic rRNAs, with human 28S rRNA containing only two m^5C nucleotides at position 4413/4 and 3761 (Maden, B.E. 1988) and none in 18S rRNA (Maden, B. E. 1986).

There are 28 known m^5C sites in human tRNA (Jühling et al., 2009), of which 27 have been verified by next-generation sequencing (Squires et al., 2012). For the best-studied eukaryotic tRNAs, m^5C residues are clustered in the junction between the variable region and TC-stem, with positions 48 and 49 the most frequently modified. Other locations in the anticodon loop (positions 34 and 38) have also been identified. Higher eukaryotes frequently have an additional m^5C residue in the tRNA acceptor stem at position 72.

These modifications are in part written by DNA methyltransferase 2 (DNMT2), a methyltransferase enzyme that was first described as methylating the cytosine at position 38 in $tRNA^{Asp}$, and NSUN2 that methylates the 5^{th} position of cytosines in tRNAs, ncRNAs, and vault-associated RNAs. However, there are a wider range of methyltransferases, including NSUN1 (NOP2), NSUN3, NSUN4, NSUN5, NSUN6 and NSUN7, that are known to methylate different RNAs in distinct cellular localizations (Bohnsack et al., 2019).

There is now evidence pointing to a critical role for tRNA and rRNA modifications in cellular responses to stimuli, demonstrating roles in tRNA stability (Alexandrov et al., 2006; Motorin and Helm, 2010), cellular stress responses (Begley et al., 2007; Netzer et al., 2009;

Thompson and Parker, 2009) and cell growth (Emilsson et al., 1992). In general, tRNA modifications enhance ribosome binding affinity, reduce misreading and modulate frame-shifting, all of which affect the rate and fidelity of translation (Chan et al., 2012). As most tRNA m^5C sites are located around the variable region and anticodon loop, this methylation stabilizes tRNA secondary structure, affects aminoacylation and codon recognition, and confers metabolic stability (Liebers et al., 2014).

Additionally, ribosomal RNA methylation has been shown to be involved in translational fidelity and tRNA recognition (Liebers et al., 2014). Loss of vault RNA methylation in NSun2-deficient mice causes aberrant vault processing into Argonaute-associated small RNAs, as well as aberrant expression of several mRNAs that are the putative targets of vault-derived small RNAs (Hussain et al., 2013). These findings provided the first mechanistic insight into the role of RNA methylation in gene regulation. DNMT2-mediated methylation has also been shown to protect substrate tRNAs against endonucleolytic cleavage (Liebers et al., 2014), with lack of Dnmt2 and NSun2 and subsequent tRNA hypomethylation associated with decreased tRNA levels and a significant reduction in protein translation rates (Liebers et al., 2014).

3. Epitranscriptomics in disease

3.1 Cancer

As with the field of epigenetics, much of the early work in epitranscriptomics has related to malignancies, with identification of both disruption of normal RNA modification patterns and changes in the expression of their writers, erasers and readers. In particular, the m^6A modification has been revealed to have a key role in promoting the stemness of malignant cells.

The METTL3/METTL14-mediated addition of m^6A to pluripotency-associated mRNAs results in their breakdown, and thereby promotes cellular differentiation of mouse embryonic stem cells (Geula et al., 2015). However, in contrast to this, METTL3 and METTL14 are very highly expressed in haematopoietic stem cells and show reduced expression in more differentiated myeloid cells (Barbieri et al., 2017). The expression and activity of these m^6A writers is also high in acute myeloid leukaemia (AML) and appears to have a key in maintaining the stemness of the malignant cells, with knockdown of these genes resulting in growth arrest via changes in the m^6A content of transcripts associated with the cell cycle and c-myc (Barbieri et al., 2017). The m^6A eraser FTO is also up-regulated and plays a critical oncogenic role in AML, in part by demethylating and thereby down-regulating the tumour-suppressors ASB2 and RARA (Li et al., 2017b). Together these studies demonstrate the complexity in understanding the impact of RNA modifications and the dysregulation of RNA methylation machinery, with transcript-specific effects.

Similar to AML, m^6A writers and erasers are implicated in the maintenance of stemness in glioblastoma. The m^6A eraser ALKBH5 is highly expressed in glioblastoma stem-like cells (GSCs), and its silencing suppresses proliferation of patient-derived GSCs (Li et al., 2017a). This is supported by evidence that knockdown of the eraser FTO suppresses tumour progression, while the knockdown of the writers METTL3 and METTL14 promotes human GSC growth, self-renewal and tumorigenesis, (Cui et al., 2017). These associations appear to be driven by the regulation of m^6A near the start and stop codons of transcripts relating to GSC self-renewal, such as ADAM19.

The tumour microenvironment may influence the cancer epitranscriptome. Hypoxia is associated with increased expression of *ALKBH5,* leading to demethylation and increased stability of the NANOG transcription factor that promotes pluripotency (Zhang et al., 2016),

and induces the ZNF217-mediated inhibition of the RNA methylation writer complex (Zhang et al., 2016).

There is also evidence for disruption of m^5C in malignancies. The NSUN2-mediated methylation of oncogenic mRNAs in bladder tumours promotes their stability and translation, thereby facilitating progression of the disease (Chen et al., 2019b), while *NSUN2* expression is up-regulated in breast cancer and promoters cell proliferation and invasion (Yi et al., 2017). The cause of NSUN2 up-regulation in solid tumours such as colorectal and oral cancers can be increased copy number (Okamoto et al., 2012). There is already emerging evidence for an impact of RNA m^5C on drug sensitivity (Cheng et al., 2018), suggesting that this may be a fruitful avenue of research in the near future.

3.2 Non-malignant disease

There is more limited evidence for changes in RNA m^5C methylation in non-malignant diseases, but this is likely to change soon as the field becomes more established. Most of the evidence to date comes from analysis of genetic variant within genes encoding m^5C writers. Several studies have demonstrated that *DNMT2* mutations or knockdown are implicated in neurodevelopmental and neuronal disorders in zebrafish, drosophila, yeast (Angelova et al., 2018). In humans, studies have identified polymorphisms in *DNMT2* that are associated with spina bifida (Franke et al., 2009), and mutations in *NSUN2* and aberrant methylation of tRNAs associated with intellectual disability and Dubowitz-like syndrome (Abbasi-Moheb et al., 2012; Blanco et al., 2014; Huber et al., 2011; Martinez et al., 2012). However, there is currently a lack of data regarding the functional impact of such genetic variants and somatic mutations. One of the key challenges in the coming years will be to establish what these consequences are and how they relate to the pathology of these conditions.

4. Environmental exposures

As evidence is acquired for disruption of RNA modification processes in a range of diseases and disorders, there will undoubtedly be increasing interest in the impact of environmental exposures upon the epitranscriptome and how they may mediate disease risk. Here, we will review the current evidence for the effect of different exposures on RNA m^6A, m^1A and m^5C. In a number of cases, this evidence is comprised of altered expression of RNA methylation writers, erasers and readers as identified through gene expression microarrays, without further examination of functional impact. Several interactions including gene/protein interactions, chemical–disease and gene-disease relationships (http://ctdbase.org/) were reviewed by means of publicly available data from the Comparative Toxicogenomics Database (CTD) supported by the National Institute of Environmental Health Sciences (NIEHS) (Davis et al., 2018). We included all types of studies (cell culture, animal models, and human) that were related to changes in gene expression and/or DNA methylation in response to environmental exposures. We included studies that provided significant results for genes encoding RNA methylation modulators (i.e. writers, erasers and readers) for m^6A, m^1A, and m^5C.

4.1 Benzo(a)pyrene

Polycyclic aromatic hydrocarbons (PAHs) are formed after the incomplete combustion of organic materials, and many are known as carcinogenic, toxic, mutagenic, teratogenic, and immune system suppressors. One such compound, benzo[a]pyrene (BaP), is a mutagenic carcinogen that is ubiquitous in the environment. Most studies to date have demonstrated an effect of BaP on the expression of RNA m^6A modification enzymes through *in vitro* and *in vivo* studies (summarized in Table 1). These studies have typically reported increased expression of the writers METTL3 and WTAP (Hooven and Baird, 2008; Jennen et al., 2010;

Kerley-Hamilton et al., 2012; Magkoufopoulou et al., 2011; Malik et al., 2012; Mathijs et al., 2009; Perez et al., 2008; Qiu et al., 2011; Shi et al., 2010; Souza et al., 2016) and the readers HNRNPC, HNRNPA2B1 and YTHDF2 (Hooven and Baird, 2008; Jennen et al., 2010; Kerley-Hamilton et al., 2012; Magkoufopoulou et al., 2011; Malik et al., 2012; Mathijs et al., 2009; Perez et al., 2008; Qiu et al., 2011; Shi et al., 2010; Souza et al., 2016), while reporting reduced expression of the eraser FTO (Hooven and Baird, 2008; Jennen et al., 2010; Kerley-Hamilton et al., 2012; Magkoufopoulou et al., 2011; Malik et al., 2012; Mathijs et al., 2009; Perez et al., 2008; Qiu et al., 2011; Shi et al., 2010; Souza et al., 2016). While this suggests that there may be increased abundance of m^6A in response to BaP exposure, there is currently no data on the direct measurement of this.

Similarly, there is evidence for altered expression of m^1A and m^5C modifier enzymes in response to BaP exposure, but no data directly measuring the functional consequences of this. Two studies have reported altered expression of TRMT6, TRMT10C, and TRMT61B following BaP exposure (Kerley-Hamilton et al., 2012; Magkoufopoulou et al., 2011). Interestingly, Severson and colleagues have also demonstrated that BaP exposure induces mutations in the *TRMT6* gene, amongst others, although the functional consequences of this were not explored (Severson et al., 2014). There is conflicting evidence for the effect of BaP upon expression of m^5C writers, with increased expression of NOP2 and decreased expression of NSUN4 having been reported (Mathijs et al., 2009; Souza et al., 2016).

4.2 Aflatoxin B1

Two Aspergillus species produce aflatoxins, which are have been declared as Group 1 carcinogens in humans by IARC (IARC, 1993). The liver is the main target organ of aflatoxins, and aflatoxin B1 exposure has been shown to downregulate expression of the m^6A eraser FTO in primary human hepatocytes (Rieswijk et al., 2016). A number of m^1A

modifiers also show differential expression, including increased expression of TRMT6 in the HepaRG cell line (Josse et al., 2012) and upregulation of ALKBH1 and TRMT61B in primary mouse hepatocytes (Mathijs et al., 2009). The NOP2 m^5C writer has also been reported to be upregulated (Rieswijk et al., 2016). However, as with BaP, to date there have been no studies measuring the impact of these changes upon RNA modifications.

4.3 Bisphenol A

Bisphenol A (BPA) is an endocrine-disrupting chemical with oestrogenic properties (Beausoleil et al., 2018). BPA is used in many common products including plastics and bottles, food and beverage cans, and sports equipment. There is evidence for disruption of RNA modifier genes in response to BPA exposure (summarised in Table 2), but these studies have reported contrasting findings. Importantly, BPA is one of the few exposures for which an impact upon RNA modifications has been demonstrated.

Mahemuti and colleagues (Mahemuti et al., 2018) provided evidence for an effect in human-derived material, reporting decreased expression of the m^6A, m^1A and m^5C writers METTL3, TRTM6 and NSUN2 and decreased expression of the m^6A readers HNRNPC and HNRNPA2B1 in cultured lung fibroblasts. These results may imply wide-ranging effects upon RNA modifications in the cells, on account of down-regulation of writers of three different modifications. However, in contrast to this, Ali and colleagues (Ali et al., 2014) have reported upregulation of the writers Mettl3, Wtap, Trmt6, Trmt61a and Nsun2 and the readers Hnrnpc and Ythdf2 in rat testes, along with decreased expression of the erasers Fto and Alkbh1, suggesting a potential increase in m^6A abundance and utilisation in the tissue. Upregulation of the readers Hnrnpa2b1 and Ythdf1 have also been reported in rat heart and breast tissue (Ali et al., 2014; Jadhav et al., 2017; Ljunggren et al., 2016; Mahemuti et al., 2018; Tait et al., 2015). Crucially, however, we have provided evidence that exposure to BPA

is associated with decreased global m^6A in the A549 human lung adenocarcinoma cell line (Cayir et al., 2019), thereby potentially supporting the findings of Mahemuti and colleagues in cultured cells from the same tissue. Our study is, to date, one of the very few to demonstrate an impact of environmental exposures upon the epitranscriptome. Nonetheless, it remains to be established whether the conflicting findings represent tissue-specific (e.g. hormone-sensitive tissues) or species-specific effects, or indeed whether they are the product of study design (*in vitro* versus *in vivo*).

4.4 Tetrachlorodibenzo-p-dioxin

Tetrachlorodibenzo-p-dioxin (TCDD) was classified as Group 1 carcinogen in humans by IARC in 1997 (Steenland et al., 2004), produced as a by-product of the burning of organic material. Work in animal models has demonstrated that TCDD exposure is associated with increased expression of the m^6A writer Wtap in the mouse brain (Gohlke et al., 2009; Rasinger et al., 2014) and the m^5C writer Nsun2 in rat and mouse liver (Boutros et al., 2008), but decreased expression of the m^6A reader Hnrnpc in mice uterine tissue (Gohlke et al., 2009; Neri et al., 2011; Rasinger et al., 2014; Thornley et al., 2011). In support of this, WTAP and METTL3 have been shown to be overexpressed in cultured human hepatocytes following TCDD exposure (Gohlke et al., 2009; Neri et al., 2011; Rasinger et al., 2014; Thornley et al., 2011). Together this may suggest promotion of m^6A methylation of RNA in response to exposure, but once again data is lacking to support such a hypothesis. In contrast to this, there have been conflicting findings regarding m^1A modifiers, with Boutros and colleagues reporting reduced expression of both Trmt10c and Alkbh1 in rat liver (Boutros et al., 2011), while Lee *et al* reported increased Alkbh1 expression in mouse liver (Lee et al., 2015). As such, no inferences can be made at this point regarding the impact on m^1A and m^5C.

4.5 Pesticides

Pesticides have been associated with changes in the male reproductive system through affecting genomic imprinting in sperm (Stouder and Paoloni-Giacobino, 2010) and transgenerational disorders due to other epigenetic alterations (Anway et al., 2006), and even affecting spermatogenic capacity in adults who were exposed during foetal development (Uzumcu et al., 2004). Furthermore, exposure has been linked to cancer, neurodegenerative disorders and endocrine disruption (Bolognesi et al., 2011; Mostafalou and Abdollahi, 2013; Owens et al., 2010). Four pesticides whose impact upon the epitranscriptome have been analysed are atrazine, diuron, diazinon, and vinclozolin (summarised in Table 3).

Atrazine is one of the most widely used herbicides. However, early studies have not clarified a clear role for this pesticide in disruption of RNA modifications. In cell lines and primary cells, studies have variously reported atrazine as downregulating the expression of m^6A, m^1A and m^5C writers and erasers and upregulating readers (Koo et al., 2012; Midic et al., 2016; Wirbisky et al., 2015). However, work in zebrafish embryos has contradicted these findings, with increased expression of METTL14, ALKBH5 and TRMT6 (Wirbisky et al., 2015). Another herbicide, diuron, has been reported to increase expression of the m^5C writer Nsun4 in the rat urinary bladder (Ihlaseh et al., 2011). With these studies presenting contrasting findings, and often simultaneously reporting increased or decreased expression of both writers and erasers, few conclusions can be drawn at this time.

Diazinon is an organophosphate insecticide that is commonly used by homeowners, but which was classified by IARC in 2015 as "probably carcinogenic to humans" (Group 2A) (Guyton et al., 2015). To date, there have been two studies regarding the regulation of m^5C modification-related genes with diazinon exposure. While *in vitro* work has revealed promoter hypermethylation of the writer *NSUN5* in the K562 cell line (Zhang et al., 2012), *NSUN4* expression has been shown to be increased in SH-SY5Y cells (Koo et al., 2012).

These results are not necessarily contradictory, as while promoter methylation is commonly associated with gene silencing there was no evaluation of its impact in the K562 cells, and DNA methylation can also be associated with increased gene expression.

In our aforementioned study of the impact of carcinogens and endocrine disruptors on m^6A methylation in A549 cells (Cayir et al., 2019), we demonstrated that vinclozolin, a widely used pesticide, decreased m^6A in a dose-response manner. This to date remains the only direct evidence for an effect of pesticides upon the epitranscriptome. It may be seen as supported by evidence of decreased expression of the m^6A reader Hnrnpa2b1 in the rat ovary (Nilsson et al., 2012), but other work from the same group has revealed increased expression of the same gene in rat testes following the same exposure (Guerrero-Bosagna et al., 2013). This suggests a tissue-specific effect of the pesticide and underlines the need for caution when extrapolating results from cell lines. While these studies suggest that vinclozolin exposure warrants further examination, it is still to be established whether m^6A is disrupted in exposed organisms. In addition to m^6A, there is some limited evidence that modifiers of m^1A may also be affected, with increased expression of tRNA methyltransferases in a variety of different rat tissues (Skinner et al., 2012), but decreased in the brain (Crews et al., 2012).

4.6 Metals

Metals are among the most widely studied exposures in relation to the expression of RNA modifier genes. The findings of studies examining a range of metal exposures are summarised in Table 4.

Arsenic is classified as carcinogenic to humans by IARC (IARC, 2004) and is associated with malignancies of lung, skin and bladder, in addition to being implicated in cardiovascular diseases (Navas-Acien et al., 2005) and neurodegenerative disease (Vahidnia et al., 2007). Arsenic compounds can be found in the air, soil and water, and exposure to

them is known to lead to DNA methylation changes (Jomova et al., 2011). There is now emerging evidence from *in vitro* studies for an impact on the epitranscriptome as well. We have recently demonstrated that exposure to sodium arsenite leads to a dose-response reduction in m^6A in A549 lung adenocarcinoma cells (Cayir et al., 2019). This is supported by gene expression studies conducted elsewhere that have identified decreased METTL3 expression in NB4 leukaemic cells (Zheng et al., 2005) and decreased WTAP expression in non-malignant lung epithelial cells (Clancy et al., 2012). Interestingly, the level of arsenite exposure may impact upon m^6A levels, as Chen and colleagues reported an increase (and increased expression of m^6A writers) with low levels of exposure but decreased m^6A in response to higher exposures (Chen et al., 2019a). The duration of exposure may also be crucial, as extended exposure (13 weeks) has been reported to increase m^6A levels in bronchial epithelial cells (Gu et al., 2018), and has also been reported in neuronal cells following six months of exposure (Bai et al., 2018). While sodium arsenite is perhaps the best studied environmental exposure in relation to the epitranscriptome, there are already apparently-conflicting results that demand further analysis. Nonetheless, the observation of changes in global RNA m^6A levels in these *in vitro* studies marks sodium arsenite as a potentially promising area of future research. Work will need to be conducted in animal models and human population studies to confirm these *in vitro* findings, and transcript-specific analysis will need to be performed to understand the functional consequences.

Another potentially fruitful avenue of future research is in the effects of copper exposure. Independent *in vitro* studies from two groups using human liver and lung cell lines have similarly revealed increased expression of the m^6A writer METTL14 (Song et al., 2009), while elsewhere the reader HNRNPA2B1 has been shown to be similarly upregulated (Armendariz et al., 2006). The latter is supported by *in vivo* findings of increased *Hnrnpa2b1* expression in mouse liver (Burkhead et al., 2011). The impact upon m^1A is less clear, as Song

and colleagues reported decreased expression of an m^1A writer (TRMT61B) and eraser (ALKBH1) in the same study (Song et al., 2009).

In addition to these studies with arsenic and copper, there is more limited *in vitro* evidence for an impact of cobaltous chloride (Hang et al., 2009; Permenter et al., 2013) and potassium chromate (VI) (Wu et al., 2012) upon m^6A erasers and readers, while studies in zebrafish have revealed decreased expression of the m^1A eraser ALKBH3 in response to methylmercury chloride (Ho et al., 2013; Yang et al., 2007) and increased expression of the m^5C writer NOP2 (Hussainzada et al., 2014). There has been very little study in human populations, not only for metals but for most exposure types, yet there is emerging evidence from three independent studies that nickel exposure is associated with increased expression of the m^5C writers NOP2 and NSUN4 (da Rosa et al., 2015; Dhingra et al., 2014), and that NSUN3 is upregulated in peripheral blood with lead exposure (LaBreche et al., 2011).

4.7 Nanoparticles

Early work on carbon nanotube exposure may suggest an increase in m^6A and m^1A abundance and utilisation. Gene expression profiling by microarray has revealed increased expression of Hnrnpa2b1, Trmt6 and Trmt61a in mouse liver (Poulsen et al., 2015a) and lung (Poulsen et al., 2015b), with upregulation of HNRNPA2B1 similarly reported in exposed non-malignant lung epithelial cells (Park et al., 2014) and observed at the protein level in a human liver cell line (Yuan et al., 2011).

4.8 Others

It should be noted that while the above represent some of the most widely studied exposures, there are of course others of emerging interest, with asbestos having been observed to affect the expression of WTAP in human pleural mesothelial cells (Dragon et al., 2015) and the

heterocyclic compound furan has been shown to induce the expression of Trmt61a in rat liver (Dong et al., 2016). It is expected that the coming years will see both strengthened examination of the epitranscriptome (as opposed to the expression of its regulators) and increased breadth in the range of exposures examined.

5. Conclusions and future directions

Epitranscriptomics is an emerging field within molecular biology that is likely to see a sharp increase in interest in the next five years. As with the early studies of DNA methylation, much of the pioneering work has been performed in relation to human disease, and especially cancer. These studies have sought to understand the aberrant expression and activity of RNA methylation modifiers in malignancies and neurodevelopmental disorders, and to elucidate the subsequent impact on the translation of genes with key roles in their pathology. By contrast, environmental epitranscriptomics is at a very embryonic stage. To date there have been very few studies directly focused upon the expression of RNA methylation modifiers, and even fewer that have demonstrated an impact upon m^6A, m^1A or m^5C modifications. Many of the studies we have presented within this review have reported changes in the expression of RNA methylation writers, erasers and readers in response to a range of exposures, but often as part of transcriptome-wide analysis and without detailed follow-up. Nonetheless, our review of the literature has indeed identified evidence for such changes, and we hope this will help to identify those exposures with the strongest evidence for an effect on the epitranscriptome. In our own work, we have demonstrated a dose-response with changes in global m^6A levels in response to BPA, sodium arsenite, vinclozolin and particulate matter exposures (Cayir et al., 2019). In particular, our observation with sodium arsenite exposure is

supported by expression-based studies conducted elsewhere that have reported decreased expression of m^6A writers (Clancy et al., 2012; Zheng et al., 2005). BPA also appears to warrant further attention, with tissue-specific effects likely to be seen. Although there is no data yet on changes to RNA modifications, there is evidence to suggest that the carcinogen BaP may impact upon the epitranscriptome, with several studies reporting concordant findings of increased expression of m^6A writers and readers, and decreased expression of erasers.

Clearly, there is a need to address the dearth of studies directly measuring RNA modifications in response to exposures. However, such studies will be very challenging to perform, and this represents a significant barrier to progress within the field. Early work on malignancies such as AML and glioblastoma demonstrated transcript-specific alteration of RNA modifications, leading to up-regulation of transcripts associated with cell cycle progression and pro-survival signalling. While analysis of global m^6A, m^1A and m^5C levels may offer some insight into the biological response to exposures, it will not be able to reveal key genes and pathways required to truly elucidate their impact; it is analogous to measuring global changes in DNA methylation and making inferences from this, rather than being able to perform gene-specific analysis by microarray or bisulfite-sequencing. Transcriptome-wide analysis of RNA modifications by techniques such as methylated RNA immunoprecipitation (MeRIP) sequencing are therefore highly preferable, but they are expensive and require large quantities of purified mRNA (typically 300 μg of starting total RNA) that exceed what can be afforded by cohort studies. Furthermore, some have questioned the reproducibility of studies using such approaches (McIntyre et al., 2020). These issues currently prohibit the application of transcript-specific analysis to human population studies. One potential solution to this is to marry *in vitro* or *in vitro* studies incorporating techniques such as MeRIP-Seq with follow-up in human subjects. Work performed in cell lines or, preferably, animal models could be used

to identify those transcripts (or other RNA molecules) showing alteration in their modifications in response to exposures, with the identified transcripts taken forward for target-specific analysis by more focussed and cost-effective approaches.

So, where are we now and where will we go? There is some early evidence that environmental exposures influence the expression of RNA methylation modulators, but largely without further analysis of the epitranscriptome. We suggest that the key next step is to perform transcriptome-wide analysis of RNA modifications in animal models under exposure to reveal targets for follow-up in human population studies. Global analysis of m^6A, m^1A and m^5C may still serve as proof-of-principle and support the development of future studies, but it is unlikely to provide lasting insight into disease processes.

Acknowledgments

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK-115Z684) and by COST Action CA16120. All authors read and approved the final manuscript.

Authors' contributions

AC gathered the results of the different studies. AC and TMB wrote the manuscript, which HMB revised. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

References

Abbasi-Moheb, L., et al., 2012. Mutations in NSUN2 cause autosomal-recessive intellectual disability. The American Journal of Human Genetics. 90**,** 847-855.

Alexandrov, A., et al., 2006. Rapid tRNA decay can result from lack of nonessential modifications. Molecular cell. 21**,** 87-96.

Ali, S., et al., 2014. Exposure to low-dose bisphenol A impairs meiosis in the rat seminiferous tubule culture model: a physiotoxicogenomic approach. PloS one. 9**,** e106245.

Angelova, M. T., et al., 2018. The emerging field of epitranscriptomics in neurodevelopmental and neuronal disorders. Frontiers in bioengineering and biotechnology. 6**,** 46.

Anway, M. D., et al., 2006. Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. Endocrinology. 147**,** 5515-5523.

Armendariz, A. D., et al., 2006. Gene expression profiling in wild-type and metallothionein mutant fibroblast cell lines. Biological research. 39**,** 125-142.

Bai, L., et al., 2018. m6A demethylase FTO regulates dopaminergic neurotransmission deficits caused by arsenite. Toxicological Sciences. 165**,** 431-446.

Barbieri, I., et al., 2017. Promoter-bound METTL3 maintains myeloid leukaemia by m 6 A-dependent translation control. Nature. 552**,** 126-131.

Beausoleil, C., et al., 2018. Regulatory identification of BPA as an endocrine disruptor: context and methodology. Molecular and Cellular Endocrinology. 475**,** 4-9.

Begley, U., et al., 2007. Trm9-catalyzed tRNA modifications link translation to the DNA damage response. Molecular cell. 28**,** 860-870.

Blanco, S., et al., 2014. Aberrant methylation of tRNAs links cellular stress to neuro□ developmental disorders. The EMBO journal. 33**,** 2020-2039.

Boccaletto, P., et al., 2018. MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic acids research. 46**,** D303-D307.

Bohnsack, K. E., et al., 2019. Eukaryotic 5-methylcytosine (m5C) RNA methyltransferases: mechanisms, cellular functions, and links to disease. Genes. 10**,** 102.

Bolognesi, C., et al., 2011. Micronuclei and pesticide exposure. Mutagenesis. 26**,** 19-26.

Boutros, P. C., et al., 2008. Transcriptomic responses to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) in liver: comparison of rat and mouse. BMC genomics. 9**,** 419.

Boutros, P. C., et al., 2011. Hepatic transcriptomic responses to TCDD in dioxin-sensitive and dioxin-resistant rats during the onset of toxicity. Toxicology and applied pharmacology. 251**,** 119-129.

Burkhead, J. L., et al., 2011. Elevated copper remodels hepatic RNA processing machinery in the mouse model of Wilson's disease. Journal of molecular biology. 406**,** 44-58.

Cao, G., et al., 2016. Recent advances in dynamic m6A RNA modification. Open biology. 6**,** 160003.

Cayir, A., et al., 2019. Exposure to environmental toxicants reduces global N6-methyladenosine RNA methylation and alters expression of RNA methylation modulator genes. Environmental research. 175**,** 228-234.

Chan, C. T., et al., 2012. Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. Nature communications. 3**,** 937.

Chen, H., et al., 2019a. Changes of RNA N6-methyladenosine in the hormesis effect induced by arsenite on human keratinocyte cells. Toxicology in Vitro. 56**,** 84-92.

Chen, X., et al., 2019b. 5-methylcytosine promotes pathogenesis of bladder cancer through stabilizing mRNAs. Nature cell biology. 21**,** 978-990.

Cheng, J. X., et al., 2018. Author Correction: RNA cytosine methylation and methyltransferases mediate chromatin organization and 5-azacytidine response and resistance in leukaemia. Nature communications. 9**,** 1-1.

Clancy, H. A., et al., 2012. Gene expression changes in human lung cells exposed to arsenic, chromium, nickel or vanadium indicate the first steps in cancer. Metallomics. 4**,** 784-793.

Crews, D., et al., 2012. Epigenetic transgenerational inheritance of altered stress responses. Proceedings of the National Academy of Sciences. 109**,** 9143-9148.

Cui, Q., et al., 2017. m 6 A RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. Cell reports. 18**,** 2622-2634.

da Rosa, J. C., et al., 2015. Patients with atopic dermatitis have attenuated and distinct contact hypersensitivity responses to common allergens in skin. Journal of Allergy and Clinical Immunology. 135**,** 712-720.

Davis, A. P., et al., 2018. The comparative toxicogenomics database: update 2019. Nucleic acids research. 47**,** D948-D954.

Davis, F. F., Allen, F. W., 1957. Ribonucleic acids from yeast which contain a fifth nucleotide. Journal of Biological Chemistry. 227**,** 907-915.

Dhingra, N., et al., 2014. Molecular profiling of contact dermatitis skin identifies allergen-dependent differences in immune response. Journal of Allergy and Clinical Immunology. 134**,** 362-372.

Dina, C., et al., 2007. Variation in FTO contributes to childhood obesity and severe adult obesity. Nature genetics. 39**,** 724.

Dominissini, D., et al., 2012. Topology of the human and mouse m 6 A RNA methylomes revealed by m 6 A-seq. Nature. 485**,** 201.

Dominissini, D., et al., 2016. The dynamic N 1-methyladenosine methylome in eukaryotic messenger RNA. Nature. 530**,** 441.

Dong, H., et al., 2016. Toxicogenomic assessment of liver responses following subchronic exposure to furan in Fischer F344 rats. Archives of toxicology. 90**,** 1351-1367.

Dragon, J., et al., 2015. Differential susceptibility of human pleural and peritoneal mesothelial cells to asbestos exposure. Journal of cellular biochemistry. 116**,** 1540-1552.

Dunn, D., 1961. The occurence of 1-methyladenine in ribonucleic acid. Biochimica et biophysica acta. 46**,** 198-200.

Emilsson, V., et al., 1992. Thiolation of transfer RNA in Escherichia coli varies with growth rate. Nucleic acids research. 20**,** 4499-4505.

Franke, B., et al., 2009. An association study of 45 folate□related genes in spina bifida: Involvement of cubilin (CUBN) and tRNA aspartic acid methyltransferase 1

(TRDMT1). Birth Defects Research Part A: Clinical and Molecular Teratology. 85**,** 216-226.

Frayling, T. M., 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**,** 889-894.

Geula, S., et al., 2015. m6A mRNA methylation facilitates resolution of naïve pluripotency toward differentiation. Science. 347**,** 1002-1006.

Gilbert, W. V., et al., 2016. Messenger RNA modifications: Form, distribution, and function. Science. 352**,** 1408-1412.

Gohlke, J. M., et al., 2009. AhR-mediated gene expression in the developing mouse telencephalon. Reproductive toxicology. 28**,** 321-328.

Gu, S., et al., 2018. N6-methyladenosine mediates the cellular proliferation and apoptosis via microRNAs in arsenite-transformed cells. Toxicology letters. 292**,** 1-11.

Guerrero-Bosagna, C., et al., 2013. Environmentally induced epigenetic transgenerational inheritance of altered Sertoli cell transcriptome and epigenome: molecular etiology of male infertility. PloS one. 8**,** e59922.

Guyton, K. Z., et al., 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. The Lancet Oncology. 16**,** 490-491.

Hanagata, N., et al., 2011. Molecular responses of human lung epithelial cells to the toxicity of copper oxide nanoparticles inferred from whole genome expression analysis. ACS nano. 5**,** 9326-9338.

Hang, X., et al., 2009. Transcription and splicing regulation in human umbilical vein endothelial cells under hypoxic stress conditions by exon array. BMC genomics. 10**,** 126.

He, C., 2010. Grand challenge commentary: RNA epigenetics? Nature chemical biology. 6**,** 863.

Ho, N. Y., et al., 2013. Gene responses in the central nervous system of zebrafish embryos exposed to the neurotoxicant methyl mercury. Environmental science & technology. 47**,** 3316-3325.

Hooven, L. A., Baird, W. M., 2008. Proteomic analysis of MCF-7 cells treated with benzo [a] pyrene, dibenzo [a, l] pyrene, coal tar extract, and diesel exhaust extract. Toxicology. 249**,** 1-10.

Huber, R. S., et al., 2011. Dubowitz syndrome: a review and implications for cognitive, behavioral, and psychological features. Journal of clinical medicine research. 3**,** 147.

Hussain, S., et al., 2013. NSun2-mediated cytosine-5 methylation of vault noncoding RNA determines its processing into regulatory small RNAs. Cell reports. 4**,** 255-261.

Hussainzada, N., et al., 2014. Whole adult organism transcriptional profiling of acute metal exposures in male zebrafish. BMC Pharmacology and Toxicology. 15**,** 15.

IARC, 1993. Aflatoxins,IARC monographs on the evaluation of carcinogenic risks to humans. Lyon: International Agency for Research on Cancer. 245-395.

IARC, 2004. Some drinking-water disinfectants and contaminants, including arsenic. IARC.

Ihlaseh, S. M., et al., 2011. Transcriptional profile of diuron-induced toxicity on the urinary bladder of male Wistar rats to inform mode of action. Toxicological Sciences. 122**,** 330-338.

Jadhav, R. R., et al., 2017. DNA methylation targets influenced by bisphenol a and/or genistein are associated with survival outcomes in breast cancer patients. Genes. 8**,** 144.

Jennen, D. G. J., et al., 2010. Comparison of HepG2 and HepaRG by whole-genome gene expression analysis for the purpose of chemical hazard identification. Toxicological sciences. 115**,** 66-79.

Jomova, K., et al., 2011. Arsenic: toxicity, oxidative stress and human disease. Journal of Applied Toxicology. 31**,** 95-107.

Josse, R., et al., 2012. Identification of early target genes of aflatoxin B1 in human hepatocytes, inter-individual variability and comparison with other genotoxic compounds. Toxicology and applied pharmacology. 258**,** 176-187.

Jühling, F., et al., 2009. tRNAdb 2009: compilation of tRNA sequences and tRNA genes. Nucleic acids research. 37**,** D159-D162.

Kerley-Hamilton, J. S., et al., 2012. Inherent and benzo [a] pyrene-induced differential aryl hydrocarbon receptor signaling greatly affects life span, atherosclerosis, cardiac gene expression, and body and heart growth in mice. Toxicological Sciences. 126**,** 391-404.

Klungland, A., Dahl, J. A., 2014. Dynamic RNA modifications in disease. Current opinion in genetics & development. 26**,** 47-52.

Koo, H.-J., et al., 2012. Endoplasmic reticulum stress impairs insulin signaling through mitochondrial damage in SH-SY5Y cells. Neurosignals. 20**,** 265-280.

LaBreche, H. G., et al., 2011. Peripheral blood signatures of lead exposure. PloS one. 6**,** e23043.

Lee, J., et al., 2015. Male and female mice show significant differences in hepatic transcriptomic response to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. BMC genomics. 16**,** 625.

Li, X., et al., 2017a. Base-resolution mapping reveals distinct m1A methylome in nuclear-and mitochondrial-encoded transcripts. Molecular cell. 68**,** 993-1005. e9.

Li, Z., et al., 2017b. FTO plays an oncogenic role in acute myeloid leukemia as a N 6-methyladenosine RNA demethylase. Cancer cell. 31**,** 127-141.

Liebers, R., et al., 2014. Epigenetic Regulation by Heritable RNA. PLoS genetics. 10**,** e1004296.

Little, N. A., et al., 2000. Identification of WTAP, a novel Wilms' tumour 1-associating protein. Human molecular genetics. 9**,** 2231-2239.

Liu, F., et al., 2016. ALKBH1-mediated tRNA demethylation regulates translation. Cell. 167**,** 816-828. e16.

Ljunggren, S. A., et al., 2016. Altered heart proteome in fructose-fed Fisher 344 rats exposed to bisphenol A. Toxicology. 347**,** 6-16.

Magkoufopoulou, C., et al., 2011. Comparison of phenotypic and transcriptomic effects of false-positive genotoxins, true genotoxins and non-genotoxins using HepG2 cells. Mutagenesis. 26**,** 593-604.

Mahemuti, L., et al., 2018. Bisphenol A induces DSB-ATM-p53 signaling leading to cell cycle arrest, senescence, autophagy, stress response, and estrogen release in human fetal lung fibroblasts. Archives of toxicology. 92**,** 1453-1469.

Malik, A. I., et al., 2012. Hepatic mRNA, microRNA, and miR□34a□Target responses in mice after 28 days exposure to doses of benzo (a) pyrene that elicit DNA damage and mutation. Environmental and molecular mutagenesis. 53**,** 10-21.

Martinez, F. J., et al., 2012. Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome. Journal of medical genetics. 49**,** 380-385.

Mathijs, K., et al., 2009. Discrimination for genotoxic and nongenotoxic carcinogens by gene expression profiling in primary mouse hepatocytes improves with exposure time. Toxicological sciences. 112**,** 374-384.

McGuinness, D. H., McGuinness, D., 2014. m6a RNA methylation: the implications for health and disease. Journal of Cancer Science and Clinical Oncology. 1.

McIntyre, A. B., et al., 2020. Limits in the detection of m 6 A changes using MeRIP/m 6 A-seq. Scientific reports. 10**,** 1-15.

Meyer, K. D., et al., 2015. 5′ UTR m6A promotes cap-independent translation. Cell. 163**,** 999-1010.

Meyer, K. D., et al., 2012. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell. 149**,** 1635-46.

Midic, U., et al., 2016. Effects of long-term endocrine disrupting compound exposure on Macaca mulatta embryonic stem cells. Reproductive Toxicology. 65**,** 382-393.

Morena, F., et al., 2018. Above the epitranscriptome: RNA modifications and stem cell identity. Genes. 9**,** 329.

Mostafalou, S., Abdollahi, M., 2013. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicology and applied pharmacology. 268**,** 157-177.

Motorin, Y., Helm, M., 2010. tRNA stabilization by modified nucleotides. Biochemistry. 49**,** 4934-4944.

Navas-Acien, A., et al., 2005. Arsenic exposure and cardiovascular disease: a systematic review of the epidemiologic evidence. American journal of epidemiology. 162**,** 1037-1049.

Neri, T., et al., 2011. The differentiation of cardiomyocytes from mouse embryonic stem cells is altered by dioxin. Toxicology letters. 202**,** 226-236.

Netzer, N., et al., 2009. Innate immune and chemically triggered oxidative stress modifies translational fidelity. Nature. 462**,** 522-526.

Nilsson, E., et al., 2012. Environmentally induced epigenetic transgenerational inheritance of ovarian disease. PloS one. 7**,** e36129.

Okamoto, M., et al., 2012. Frequent increased gene copy number and high protein expression of tRNA (cytosine-5-)-methyltransferase (NSUN2) in human cancers. DNA and cell biology. 31**,** 660-671.

Owens, K., et al., 2010. Wide range of diseases linked to pesticides. Pestic. You. 30**,** 13-21.

Park, E.-J., et al., 2014. SWCNTs induced autophagic cell death in human bronchial epithelial cells. Toxicology in Vitro. 28**,** 442-450.

Perez, D. S., et al., 2008. Gene expression changes associated with altered growth and differentiation in benzo [a] pyrene or arsenic exposed normal human epidermal keratinocytes. Journal of Applied Toxicology: An International Journal. 28**,** 491-508.

Permenter, M. G., et al., 2013. Exposure to cobalt causes transcriptomic and proteomic changes in two rat liver derived cell lines. PloS one. 8**,** e83751.

Ping, X.-L., et al., 2014. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell research. 24**,** 177.

Poulsen, S. S., et al., 2015a. Changes in cholesterol homeostasis and acute phase response link pulmonary exposure to multi-walled carbon nanotubes to risk of cardiovascular disease. Toxicology and applied pharmacology. 283**,** 210-222.

Poulsen, S. S., et al., 2015b. MWCNTs of different physicochemical properties cause similar inflammatory responses, but differences in transcriptional and histological markers of fibrosis in mouse lungs. Toxicology and applied pharmacology. 284**,** 16-32.

Qiu, C., et al., 2011. Effects of subchronic benzo (a) pyrene exposure on neurotransmitter receptor gene expression in the rats hippocampus related with spatial learning and memory change. Toxicology. 289**,** 83-90.

RajBhandary, U., et al., Nucleotide sequence studies on yeast phenylalanine sRNA. Cold Spring Harbor symposia on quantitative biology, Vol. 31. Cold Spring Harbor Laboratory Press, 1966, pp. 425-434.

Rasinger, J. D., et al., 2014. Cross-omics gene and protein expression profiling in juvenile female mice highlights disruption of calcium and zinc signalling in the brain following dietary exposure to CB-153, BDE-47, HBCD or TCDD. Toxicology. 321**,** 1-12.

Rieswijk, L., et al., 2016. Aflatoxin B1 induces persistent epigenomic effects in primary human hepatocytes associated with hepatocellular carcinoma. Toxicology. 350**,** 31-39.

Saletore, Y., et al., 2012. The birth of the Epitranscriptome: deciphering the function of RNA modifications. Genome biology. 13**,** 175.

Scuteri, A., et al., 2007. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS genetics. 3**,** e115.

Severson, P. L., et al., 2014. Exome-wide mutation profile in benzo [a] pyrene-derived post-stasis and immortal human mammary epithelial cells. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 775**,** 48-54.

Sharma, S., et al., 2013. Identification of a novel methyltransferase, Bmt2, responsible for the N-1-methyl-adenosine base modification of 25S rRNA in Saccharomyces cerevisiae. Nucleic acids research. 41**,** 5428-5443.

Shi, Z., et al., 2010. Oral benzo [a] pyrene□induced cancer: Two distinct types in different target organs depend on the mouse Cyp1 genotype. International journal of cancer. 127**,** 2334-2350.

Skinner, M. K., et al., 2012. Epigenetic transgenerational inheritance of somatic transcriptomes and epigenetic control regions. Genome biology. 13**,** R91.

Song, M. O., et al., 2009. Physiological and toxicological transcriptome changes in HepG2 cells exposed to copper. Physiological genomics. 38**,** 386-401.

Souza, T., et al., 2016. New insights into BaP-induced toxicity: role of major metabolites in transcriptomics and contribution to hepatocarcinogenesis. Archives of toxicology. 90**,** 1449-1458.

Squires, J. E., et al., 2012. Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA. Nucleic acids research. gks144.

Steenland, K., et al., 2004. Dioxin revisited: developments since the 1997 IARC classification of dioxin as a human carcinogen. Environmental health perspectives. 112**,** 1265-1268.

Stouder, C., Paoloni-Giacobino, A., 2010. Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm. Reproduction. 139**,** 373-379.

Tait, S., et al., 2015. Bisphenol A affects placental layers morphology and angiogenesis during early pregnancy phase in mice. Journal of Applied Toxicology. 35**,** 1278-1291.

Thompson, D. M., Parker, R., 2009. The RNase Rny1p cleaves tRNAs and promotes cell death during oxidative stress in Saccharomyces cerevisiae. The Journal of cell biology. 185**,** 43-50.

Thornley, J. A., et al., 2011. Differential regulation of polysome mRNA levels in mouse Hepa-1C1C7 cells exposed to dioxin. Toxicology in Vitro. 25**,** 1457-1467.

Uzumcu, M., et al., 2004. Effect of the anti-androgenic endocrine disruptor vinclozolin on embryonic testis cord formation and postnatal testis development and function. Reproductive Toxicology. 18**,** 765-774.

Vahidnia, A., et al., 2007. Arsenic neurotoxicity—a review. Human & experimental toxicology. 26**,** 823-832.

Wang, X., et al., 2015. N 6-methyladenosine modulates messenger RNA translation efficiency. Cell. 161**,** 1388-1399.

Wang, Y., et al., 2014. N 6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nature cell biology. 16**,** 191.

Wirbisky, S. E., et al., 2015. Developmental origins of neurotransmitter and transcriptome alterations in adult female zebrafish exposed to atrazine during embryogenesis. Toxicology. 333**,** 156-167.

Wu, F., et al., 2012. Epigallocatechin-3-gallate (EGCG) protects against chromate-induced toxicity in vitro. Toxicology and applied pharmacology. 258**,** 166-175.

Yang, L., et al., 2007. Transcriptional profiling reveals barcode-like toxicogenomic responses in the zebrafish embryo. Genome biology. 8**,** R227.

Yi, J., et al., 2017. Overexpression of NSUN2 by DNA hypomethylation is associated with metastatic progression in human breast cancer. Oncotarget. 8**,** 20751.

Yuan, J., et al., 2011. Cytotoxicity evaluation of oxidized single-walled carbon nanotubes and graphene oxide on human hepatoma HepG2 cells: an iTRAQ-coupled 2D LC-MS/MS proteome analysis. Toxicological Sciences. 126**,** 149-161.

Zhang, C., et al., 2016. Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m6A-demethylation of NANOG mRNA. Proceedings of the National Academy of Sciences. 113**,** E2047-E2056.

Zhang, X., et al., 2012. Genome-wide study of DNA methylation alterations in response to diazinon exposure in vitro. Environmental toxicology and pharmacology. 34**,** 959-968.

Zheng, G., et al., 2013. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Molecular cell. 49**,** 18-29.

Zheng, P.-Z., et al., 2005. Systems analysis of transcriptome and proteome in retinoic acid/arsenic trioxide-induced cell differentiation/apoptosis of promyelocytic leukemia. Proceedings of the National Academy of Sciences of the United States of America. 102**,** 7653-7658.

Table 1. Effect of Benzo(A)pyrene on RNA modification-related genes

	Type	Tissue	Genes	Analysis	↑/↓	Ref.
m^6A	*In vitro*	HepG2 Cells	METTL3	Expression	↑	(Souza et al., 2016)
	Mouse	Aorta	METTL3	Expression	↑	(Kerley-Hamilton et al., 2012)
	Mouse	Primary Mouse Hepatocytes	METTL14	Expression	↓	(Mathijs et al., 2009)
	In vitro	Human Epidermal Keratinocytes (NHEK)	WTAP	Expression	↑	(Perez et al., 2008)
	In vitro	HepG2 Cells	FTO	Expression	↓	(Souza et al., 2016)
	In vitro	HepG2	FTO	Expression	↓	(Magkoufopoulou et al., 2011)
	In vitro	HepG2 and HepaRG	FTO	Expression	↓	(Jennen et al., 2010)
	Mouse	Aorta	ALKBH5	Expression	↑	(Kerley-Hamilton et al., 2012)
	Mouse	Liver Tissue	HNRNPC	Expression	↓	(Malik et al., 2012)
	Mouse	Aorta	HNRNPC	Expression	↑	(Kerley-Hamilton et al., 2012)
	Mouse	Different Organs	HNRNPA2B1	Expression	↑	(Shi et al., 2010)
	In vitro	MCF-7 Cells	HNRNPA2B1	Expression	↑	(Hooven and Baird, 2008)
	Rat	Hippocampus	YTHDF2	Expression	↓	(Qiu et al., 2011)
	Mouse	Aorta	YTHDF2	Expression	↑	(Kerley-Hamilton et al., 2012)
m^1A	Mouse	Aorta	TRMT6	Expression	↑	(Kerley-Hamilton et al., 2012)
	In vitro	Human mammary epithelial cells (HMEC)	TRMT6	***Mutagenesis***	↑	(Severson et al., 2014)
	Mouse	Aorta	TRMT10C	Expression	↑	(Kerley-Hamilton et al., 2012)
	In vitro	HepG2 cells	TRMT61B	Expression	↓	(Magkoufopoulou et al., 2011)
m^5C	*In vitro*	HepG2 Cells	NOP2	Expression	↑	(Souza et al., 2016)
	Mouse	Primary mouse hepatocytes	NSUN4	Expression	↓	(Mathijs et al., 2009)

PAH: polycyclic aromatic hydrocarbon, Increase (↑) or decrease (↓)

Table 2. Effect of Bisphenol A on RNA modification-related genes

	Type	Tissue	Genes	Analysis	↑/↓	Ref.
m^6A	*In vitro*	Human Fetal Lung Fibroblasts	METTL3	Expression	↓	(Mahemuti et al., 2018)
	Rat	Seminiferous Tubule	METTL3	Expression	↑	(Ali et al., 2014)
	Rat	Seminiferous Tubule	WTAP	Expression	↑	(Ali et al., 2014)
	Mice	Placenta	FTO	Expression	↓	(Tait et al., 2015)
	Rat	Seminiferous Tubule	FTO	Expression	↓	(Ali et al., 2014)
	Mice	Placenta	FTO	Expression	↑	(Tait et al., 2015)
	Rat	Mammary Gland	FTO	***Methylation***	↑	(Jadhav et al., 2017)
	In vitro	Human Fetal Lung Fibroblasts	HNRNPC	Expression	↓	(Mahemuti et al., 2018)
	Rat	Seminiferous Tubule	HNRNPC	Expression	↑	(Ali et al., 2014)
	Rat	Mammary Gland	HNRNPC	***Methylation***	↑	(Jadhav et al., 2017)
	In vitro	Human Fetal Lung Fibroblasts.	HNRNPA2B1	Expression	↓	(Mahemuti et al., 2018)
	Rat*	Heart Left Ventricles	HNRNPA2B1	Expression	↑	(Ljunggren et al., 2016)
	Rat	Seminiferous Tubule	YTHDF2	Expression	↑	(Ali et al., 2014)
	Rat	Mammary Gland	YTHDF1	Expression	↑	(Jadhav et al., 2017)
m^1A	Rat	Seminiferous tubule	TRMT61A	Expression	↑	(Ali et al., 2014)
	In vitro	Human fetal lung fibroblasts	TRMT6	Expression	↓	(Mahemuti et al., 2018)
	Rat	Seminiferous tubule	TRMT6	Expression	↑	(Ali et al., 2014)
	Rat	Seminiferous tubule	TRMT10C	Expression	↑	(Ali et al., 2014)
	Rat	Seminiferous tubule	ALKBH3	Expression	↑	(Ali et al., 2014)
	Mouse	Tail tissue	ALKBH3	***Methylation***	↑	(Jadhav et al., 2017)
	Rat	Seminiferous tubule	ALKBH1	Expression	↓	(Ali et al., 2014)
m^5C	*In vitro*	Human fetal lung fibroblasts	NSUN2	Expression	↓	(Mahemuti et al., 2018)
	Rat	Seminiferous tubule	NSUN2	Expression	↑	(Ali et al., 2014)

ED: Endocrine Disruptor, **POP**: Persistent Organic Pollutants, Increase (↑) or decrease (↓)

*Fructose Co-Treated with Bisphenol A

Table 3. Effect of pesticides on RNA modification-related genes

	Type	Tissue	Genes	Analysis	↑/↓	Ref.
m^6A	Zebrafish*	Embryos	METTL14	Expression	↑	(Wirbisky et al., 2015)
	*In vitro**	Embryonic Stem Cell	ALKBH5	Expression	↓	(Midic et al., 2016)
	Zebrafish*	Embryos	ALKBH5	Expression	↑	(Wirbisky et al., 2015)
	*In vitro**	SH-SY5Y Cells	YTHDF1	Expression	↑	(Koo et al., 2012)
	Rat**	Ovary	HNRNPA2B1	Expression	↓	(Nilsson et al., 2012)
	Rat**	Sertoli Cells	HNRNPA2B1	Expression	↑	(Guerrero-Bosagna et al., 2013)
m^1A	*In vitro**	SH-SY5Y cells	ALKBH3	Expression	↓	(Koo et al., 2012)
	Zebrafish*	Embryos	TRMT6	Expression	↑	(Wirbisky et al., 2015)
	Rat**	Brain	TRMT10C	Expression	↓	(Crews et al., 2012)
	Rat**	Different tissues	TRMT10C	Expression	↑	(Skinner et al., 2012)
m^5C	*In vitro**	SH-SY5Y cells	NSUN2	Expression	↓	(Koo et al., 2012)
	*In vitro****	K562 cell	NOP2	***Methylation***	↑	(Zhang et al., 2012)
	Rat*****	Urinary bladder	NSUN4	Expression	↑	(Ihlaseh et al., 2011)

Increase (↑) or decrease (↓)

* Atrazine (herbicide), ** Vinclozolin (Fungicide), *** Diazinon, ****Diuron.

Table 4. Effect of metals on RNA modification-related genes

	Exposure	Type	Tissue	Genes	Analysis	↑/↓	Ref.
m^6A	Arsenic	*In vitro*	APL cell line NB4	METTL3	Expression	↓	(Zheng et al., 2005)
		In vitro	BEAS-2B	WTAP	Expression	↓	(Clancy et al., 2012)
	Copper Sulfate	*In vitro*	HepG2 Cells	METTL14	Expression	↑	(Song et al., 2009)
	Cupric Oxide	*In vitro*	Lung Epithelial A549 Cell	METTL14	Expression	↑	(Hanagata et al., 2011)
	Copper	*In vitro*	Fibroblast Cell	HNRNPA2B1	Expression	↑	(Armendariz et al., 2006)
		Mouse	Liver	HNRNPA2B1	Expression	↑	(Burkhead et al., 2011)
	Cobaltous Chloride	*In vitro*	Rat Liver Derived Cell Lines	ALKBH5	Expression	↑	(Permenter et al., 2013)
		In vitro	Huvecs Cells	ALKBH5	Expression	↑	(Hang et al., 2009)
	Potassium Chromate (VI)	*In vitro*	Human Normal Bronchial Epithelial BEAS-2B Cells	YTHDF1	Expression	↑	(Wu et al., 2012)
m^1A	Copper Sulfate	*In vitro*	HepG2	TRMT61B	Expression	↓	(Song et al., 2009)
		In vitro	HepG2	ALKBH1	Expression	↓	(Song et al., 2009)
	Methylmercuric chloride	Zebrafish	Embryos	ALKBH3	Expression	↓	(Ho et al., 2013)
		Zebrafish	Embryos	ALKBH3	Expression	↓	(Yang et al., 2007)
m^5C	Nickel	Human	Skin	NOP2	Expression	↑	(da Rosa et al., 2015; Dhingra et al., 2014)
	Nickel	Zebrafish	Total fish	NOP2	Expression	↑	(Hussainzada et al., 2014)
	Nickel	Human	Skin	NSUN4	Expression	↑	(da Rosa et al., 2015; Dhingra et al., 2014)
	Lead	Human	Peripheral blood	NSUN3	Expression	↑	(LaBreche et al., 2011)

Increase (↑) or decrease (↓)

Figure Captions

Figure 1: Enzymatic regulation of RNA methylation. The m6A, m1A and m5C RNA modifications are added to RNA molecular by 'writer' methyltransferases (green), remove by 'eraser' demethylases (red) and utilised by 'readers' (blue). Examples of modifications (orange circles) to mRNA and tRNA molecules are illustrated.

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: